Vascular calcification is the major cause of cardiovascular morbidity and mortality in patients with type 2 diabetes mellitus, chronic kidney disease, and in aging patients. Considerable progress has been made in the past 2 decades in understanding the molecular mechanisms of vascular calcification. Regardless of the morphology and location, most evidence indicates that vascular calcification involves an organized process recapitulating many cellular and molecular events that govern skeletal bone formation.

Although a large body of evidence shows that osteoblastic and osteoclonic cells contribute to vascular calcification, it remains unclear how osteoclasts are differentiated from their precursors and how osteoclasts play a role in calcium reabsorption in calcifying arteries. Osteoclasts develop from monocytic precursors of the hematopoietic lineage, and 2 distinct signaling systems are both necessary and sufficient for the early differentiation into multinucleated osteoclasts as demonstrated by osteopetrotic mice models with loss-of-function mutation of either gene. One is a signaling system that involves macrophage colony-stimulating factor (MCSF) and its receptor c-fms, and the other involves receptor activator of nuclear factor κB (RANK) ligand (RANKL), RANK, and osteoprotegerin (OPG), a soluble decoy receptor for RANKL. MCSF and RANKL induce osteoclast differentiation, fusion, and maturation on binding to its specific receptors, c-fms and RANK, respectively, on the surface of preosteoclastic monocytes. Increased production of RANKL in atherosclerotic lesions, particularly in endothelial cells and vascular smooth muscle cells (SMCs), has been well documented.

Studies of OPG-deficient mice provided compelling evidence for the presence of functional osteoclasts in calcified arteries. OPG-deficient mice develop extensive arterial medial calcification coinciding with multinucleated cells, which are cathepsin K–positive and tartrate-resistant acid phosphatase (TRAP)-positive, but F4/80-negative, which is typical of osteoclasts. However, immunohistochemistry of calcifying atherosclerotic lesion from humans and mice show abundant osteoclasts and relatively fewer osteoclasts. Consistent with this, RANKL expression increases, whereas OPG expression remains unchanged or reduces in calcified lesion. OPG treatment reduces vascular calcification without affecting atherosclerosis in low-density lipoprotein receptor–deficient mice fed a high-fat diet. These results are consistent with previous findings that RANKL can directly promote osteogenic differentiation of vascular SMC and exacerbate vascular calcification and inflammation, and exogenous OPG serves a protective role against vascular calcification. Thus, it is conceivable that RANKL/RANK signaling is not sufficiently antagonized by OPG in calcifying vasculopathies, and RANKL/RANK signaling in vascular SMC is more prominent than that in preosteoclast precursors under specific conditions where atherosclerotic process progresses. Then, an important question would be which molecular mechanisms are responsible for the attenuated response of preosteoclast precursors to RANKL/RANK signaling.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Itou et al. have identified novel regulators of RANKL-induced osteoclast-like cells differentiated from murine macrophage-like cell line RAW264.7 by using a novel proteomics technology for relative quantification of proteins with liquid chromatography tandem mass tagging–based mass spectrometry analysis. Among 4244 proteins analyzed, besides several authentic osteoclast-associated markers such as cathepsin K, osteoclast-associated receptor, and TRAP 5 or acid phosphatase 5, they showed 5 novel proteins to be more abundantly expressed in mouse bone marrow–derived osteoclasts as well as in RANKL-induced osteoclasts compared with those expressed in control cells (macrophages). Among the 5 selected proteins, namely, Edil3, Ifg3, Serpinb6b, Adseverin, and cystathionine γ-lyase (CSE), the expression of Adseverin and CSE, but not others, was measurably increased at protein levels as well as at mRNA levels during osteoclastogenesis.

With regard to CSE, Itou et al pursued its physiological role in RANKL-induced osteoclastogenesis. By using silencing RNA or DL-propargylglycine, a pharmacological inhibitor for CSE, the authors showed that CSE accelerates the RANKL-induced differentiation of RAW264.7 cells into TRAP-positive, cathepsin K–positive, multinucleated osteoclast-like cells with bone resorption activity. In addition, they showed the strong expression of CSE in CD68-positive macrophages and osteoclast-associated receptor–positive osteoclast-like cells in atherosclerotic lesion in apolipoprotein E–deficient mice fed a high-fat diet.

The principal finding of this article is that the H₂S-generating enzyme CSE seems to play a significant role in osteoclastogenesis. The results in this study showed that the addition of GYY4137, an H₂S donor, promotes RANKL-induced osteoclast differentiation as demonstrated by an increase in TRAP-positive multinucleate cells with pit resorption activity in vitro.
These results represent a potential breakthrough in the understanding of the modulation of RANKL-induced osteoclastogenesis. However, it is not clear how H₂S exerts its osteoclastogenic effects on RANKL-stimulated RAW264.7 cells. Previous studies have demonstrated that H₂S is an important endogenous vasoactive factor that protects arteries from atherosclerotic damage, including inflammation, endothelial dysfunction, vascular SMC proliferation, and migration.17,18 The antiinflammatory effects of H₂S have been ascribed to its antioxidant effect because treatment of CSE-deficient, atherogenic diet–fed mice with an H₂S donor resulted in decreased oxidative stress.19 Taking these data into consideration, Itou et al alluded that the vasoprotective effect of H₂S contributes to the acceleration of osteoclast differentiation in response to the H₂S donor GYY4137.

The finding of Itou et al of the effects of H₂S on osteoclast differentiation seems to be supported by several previous studies demonstrating that increased intracellular levels of the antioxidant glutathione enhance osteoclast development and bone pit formation, and glutathione depletion by -buthionine-(S, R)-sulfoximine, a specific inhibitor of glutathione synthesis, inhibits osteoclastogenesis in RANKL-stimulated RAW264.7 cells.20 Nevertheless, there have been several studies challenging this hypothesis; reactive oxygen species produced in macrophages are essential for osteoclast differentiation21; the administration of antioxidants completely prevented bone loss in ovariectomized mice22; and several signaling components essential for RANKL-induced osteoclast differentiation are activated by reactive oxygen species, including tumor necrosis factor receptor–associated factor 6, nuclear factor κB, c-Fos, nuclear factor of activated cells, p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, extracellular signal-regulated kinase, and NADPH oxidase.23 These controversies would be reconciled by the notion that redox shift caused by the change in oxidative stress or antioxidant defense exerts its bimodal effects on cellular function depending on the cellular redox status. Thus, it is intriguing to speculate that the robustness of RANKL-induced osteoclast differentiation is dependent on the redox state of precursor cells that is regulated by H₂S generated by CSE.

What are the potential clinical implications of this study? Clearly, additional work is required to determine whether H₂S production or CSE activity is implicated in osteoclast differentiation in vivo. Moreover, it is yet to be determined whether osteoclast response to H₂S is differentially regulated between bone and vasculature. Although the relationship between osteoporosis and vascular calcification has long been known as calcification paradox, its precise molecular mechanism remains unclear. It is reassuring that calcium paradox is not merely because of calcium shift from bone to artery wall but is likely because of the differential response of both osteoblasts and osteoclasts to oxidative stress between bone and artery.24

In the past, many studies have highlighted the important role for RANK/RANKL/OPG axis for the apparently opposite regulation of calcification between 2 tissues.5–15 The report by Itou et al brings a new perspective to the regulatory mechanisms of osteoclast differentiation and may open new avenues to the identification of a promising target for the prevention and treatment of vascular calcification.

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References


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