Vascular calcification is a pathological sequence of events that has similarities to the normal physiological process of osteogenesis. It is thought to result from an imbalance in both local and systemic inhibitors and promoters, which occurs as a consequence of chronic inflammation, hyperlipidemia, and a deregulation of various bone-regulating proteins. It is common in patients with diabetes and renal failure and in the elderly. The fact that coronary artery calcification is such a major health problem and the recognition that it is highly correlated with mortality risk makes the understanding of vascular calcification a worthy and exciting area of study.

Bone Remodeling and the Role of RANKL

In normal skeletal physiology, bone deposition by osteoblasts is in balance with bone resorption by osteoclasts. RANKL (receptor activator of nuclear factor [NF]-κB ligand) and its soluble decoy receptor, osteoprotegerin (OPG), are critical regulators of bone remodeling. Binding of RANKL to its cognate receptor RANK, expressed on osteoclasts and its precursors, induces NF-κB signaling, resulting in NF-κB translocation to the nucleus with a subsequent increase in RelB levels, in turn, stimulating osteoclast differentiation (Figure, A). OPG, a decoy receptor, expressed by osteoblasts, binds with RANKL, preventing RANK (receptor activator of NF-κB) signaling and thus inhibiting osteoclastogenesis (Figure, B).

Vascular Calcification and Osteolysis

There is increasing evidence to suggest that both osteopenia and vascular calcification may be linked. Both pathological conditions are marked in patients with acute neuropathic osteoarthropathy (Charcot foot), in which osteopenia is universal and the prevalence of vascular calcification exceeds 90%. Although it is established that the RANKL/OPG signaling pathway is central to the processes regulating bone turnover in a wide variety of medical conditions, there is now a strong clinical association between coronary disease and serum OPG/RANKL levels. Therefore, RANKL/OPG are recognized as having equal importance in arterial calcification and osteolysis in bone.

The association of OPG with the prevention of vascular calcification was first realized when OPG knockout mice displayed a phenotype of calcified arteries and osteoporosis. There is also evidence for a localized increase in RANKL expression in calcified arterial tissue and a decrease in expression of OPG in calcified arteries. Local signals within the vessel wall, such as the loss of inhibitors of osteogenic differentiation (e.g., OPG, bone morphogenetic protein (BMP)2, and matrix Gla protein) and inorganic phosphate generation and activation of the wnt/β-catenin signaling, have all been shown to drive a chondro-osseous differentiation program by vascular smooth muscle cells (VSMCs).

In the case of bone deposition in the vessel wall occurring in conjunction with bone loss, with the excess calcium and mineral being released from bone, signaling events are thought to trigger an osteogenic differentiation program, whereby progenitor cells residing in the vessel wall differentiate into osteoblast-like cells, depositing a mineralized matrix (Figure, C). The origin or source of the cells involved in osteogenic differentiation remain controversial, in that there is good evidence for the presence of calcifying vascular cells within the vessel wall, although others allude to an activation and differentiation program of circulating progenitor cells.

Protection against mineralization in the vessel wall is thought to be achieved by a modulation of OPG and RANKL expression, causing inhibition of osteoclast maturation, thus preventing subsequent release of calcium and mineral from bone. Although expression levels of RANKL can often remain unchanged, the antiosteoclastogenic cytokine OPG is more often reduced in osteolysis patients or after dexamethasone treatment, thus elevating RANKL:OPG ratios. These findings would suggest that the OPG/RANKL/RANK autocrine/paracrine axis is deregulated under certain pathological situations, with a parallel osteoporotic bone loss in conjunction with bone deposition in the vasculature.

Until now, the evidence linking RANKL with vascular calcification has been circumstantial and indirect. In this issue of Circulation Research, Panizo et al report direct links between RANKL/RANK signaling, elevated levels of BMP4, and increased vascular calcification both in vitro and in vivo. They demonstrate an involvement of the IKK-α pathway, the alternative pathway of activation of NF-κB signaling, and eliminate involvement of an apoptotic process in RANKL-induced mineralization. First, using an in vitro model of calcification, they show that addition of RANKL to VSMCs in culture accelerates mineralization, as assessed by an increase in alkaline phosphatase activity, calcium incorporation, and von Kossa staining, effects that are all inhibited by addition of its soluble decoy receptor, OPG.
The authors also used small interfering (si)RNA to knock down RANK, abolishing the effect of RANKL induction of calcification, indicating a direct role of RANKL binding to RANK causing osteogenic differentiation of the VSMCs. To assess the mechanism underpinning this effect, Panizo et al investigated the downstream signaling pathways activated by RANKL, namely the NF-κB transcription factor cascade, involving the 2 kinases IKK-β and IKK-α, which are associated with the classical and alternative pathways of NF-κB activation respectively. Of note, Panizo et al demonstrate that inhibition of IKK-β, using siRNA knockdown, had no effect on calcification, whereas blocking IKK-α obliterated RANKL-induced VSMC mineralization. In addition, they showed that after incubation of the cells with RANKL, nuclear levels of RelB were elevated, adding further confirmation of the alternative pathway of NF-κB activation.

BMPs are members of the transforming growth factor-β family, initially shown to be involved in bone formation but are also implicated in vascular calcification. A striking finding of Panizo et al concerns the elevated BMP4 mRNA abundance and protein secretion resulting from RANK activation, which can be attenuated when they inhibit IKK-α. In addition, they show that addition of Noggin, an inhibitor of BMP4, blunted the increase in calcification induced by RANKL, providing evidence for the direct link between RANKL and BMP4-mediated calcification. Finally, the authors confirm RANKL-induced biomineralization in vivo, using a rat model of calcification. The use of calcitriol, a synthetic vitamin D analog, increases the absorption of calcium from renal tubular cells and also stimulates osteoclastic calcium resorption from bone. Animals with 5/6 nephrectomy show an increase in calcification, an effect enhanced by the addition of calcitriol. The interesting finding from the study by Panizo et al is the fact that local vascular expression of RANKL and BMP4 increased within the vicinity of calcification in the rat arteries, whereas systemic RANKL and OPG levels were unchanged and elevated, respectively, concluding that paracrine and autocrine regulatory pathways may differ and a disruption in the RANKL:OPG ratio has a more direct effect on the deposition of a mineralized matrix in the vessel wall than levels of RANKL per se.

**RANKL/OPG for Prevention or Treatment of Vascular Calcification: A Promising Target**

An inverse relationship between vascular calcification and bone density is very pronounced in end-stage renal disease patients (ESRD). Cross-sectional studies have demonstrated...
an inverse relationship between osteoporosis and coronary artery calcification in the general population, as well as in ESRD patients, suggesting a link between these 2 pathologies. Bisphosphonates are anticalcifying agents that have been developed as analogs of pyrophosphate and are resistant to chemical and enzymatic hydrolysis. They bind to hydroxyapatite crystals, inhibiting their formation and dissolution. Bisphosphonates are absorbed by osteoclasts, inhibiting their function. Price et al have demonstrated that the bisphosphonates alendronate and ibandronate suppressed the development of uremia-related vascular calcification in 42-day-old male rats treated with warfarin. Bisphosphonates are widely administered for treatment of osteoporosis, a condition in which the prevention of bone resorption is important (Figure, A). A recent randomized control trial has shown that denosumab, a human monoclonal antibody that specifically inhibits RANKL, by mimicking the effect of OPG, is more effective in increasing bone mineral density in postmenopausal women with osteopenia, compared to placebo or treatment with alendronate. More recent experiments in murine models of bone disease have also shown that treatment with denosumab reduced bone resorption and improved trabecular microarchitecture. The protective effects of the bisphosphonates and denosumab may be attributed to the inhibition of osteoclastogenesis, justifying support for further studies of the antiresorptive effects of molecules with anti-RANKL activity, enabling the subsequent prevention of both osteoporosis and vascular calcification.

Conclusions
In summary, the findings by Panizo et al provide new insights into the molecular pathogenesis of vascular calcification, and the data add to the interest in targeting RANKL/OPG for the potential prevention of calcium deposition in the vessel wall. Further studies are warranted to elaborate the effects of RANKL in vascular calcification and to test RANKL antagonism in appropriate animal models and in the treatment of patients with acute vascular syndromes.

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