More than a century ago, Mönckeberg\(^1\) was among the first to note that ectopic calcification may arise in the vasculature. Although this original description was of a specific form of sclerosing calcification that largely affects the vascular medial layer, we have since gone on to appreciate that generalized vascular calcification is a pervasive and likely inevitable program that is intimately entwined with aging, atherosclerosis, and cardiovascular disease. Global measures of coronary artery calcification were recently shown to independently predict both cardiovascular events and patient mortality.\(^2,3\) However, adding complexity, it is now clear that the vascular medial layer, we have since gone on to appreciate that generalized vascular calcification is a pervasive and likely inevitable program that is intimately entwined with aging, atherosclerosis, and cardiovascular disease. Global measures of coronary artery calcification were recently shown to independently predict both cardiovascular events and patient mortality.\(^2,3\) However, adding complexity, it would appear that arterial and coronary calcification serve only as markers of the overall burden of vascular disease, rather than identifying particular lesions that are likely to cause future events.\(^4\) A central conundrum in the field is the so-called calcification paradox: in many patients and rodent models, atherosclerosis occurs simultaneously with advancing vascular calcification.\(^5\) Although it has become increasingly clear that the pathways that control skeletal bone formation and density are also operative in vascular calcification,\(^5,6\) the paradox leaves open the question of whether the 2 processes may diverge at key regulatory steps or whether the differing local milieu accounts for this observation.

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This concern is relevant to a new study in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, as Byon et al\(^7\) present surprising results on the regulation of calcification by vascular smooth muscle cells (VSMCs) from *apolipoprotein E*\(^{-/-}\) mouse arteries. In the context of atherosclerosis, these cells can differentiate to osteoblast-like cells that in turn regulate vascular calcification. Byon et al agree that this calcifying activity, like the formation of skeletal bone, is dependent on the transcription factor Runx2, the master regulator of osteoblast differentiation.\(^5\) However, herein lies an intriguing twist. This body of work, along with another recent report by the same group, indicates that oxidative stress, such as locally generated H\(_2\)O\(_2\), promotes osteoblast- and osteoclast-like cell differentiation and the calcifying program coordinated by Runx2 (Figure).\(^7,8\) By contrast, in numerous other studies on osteoblasts obtained directly from the bone marrow environment, oxidative stress is remarkable as a potent inhibitor of osteoblast development and instead favors adipocyte development from the same precursors.\(^9–11\) Is the difference in outcome due to the fact that the calcifying cells studied herein were VSMCs and not osteoblasts? Do these sources of calcifying cells possess some underlying differences in regulation, and is the response to oxidative stress one of those pivotal differences? Mechanistically, in studies on authentic osteoblasts, the transcription factor c-Maf is crucial for osteoblast differentiation, and its expression is inhibited by oxidative stress.\(^10,11\) There are currently no studies on the role of c-Maf and vascular calcification, highlighting an important future direction.

The current work by Byon et al also focuses on the accumulation of multinucleated osteoclast-like macrophages in atherosclerotic plaques.\(^7\) First, the authors show that Runx2 binds to the RANKL promoter and controls its transcription, in concert with H\(_2\)O\(_2\), as discussed above, in VSMCs. Going against prior reports,\(^12,13\) in the hands of Byon et al, RANKL did not induce calcification by VSMCs. Rather, consistent with the well-documented role of RANKL in osteoclast differentiation, RANKL promoted the migration and differentiation of macrophages into osteoclast-like cells. We believe it is unlikely that RANKL recruits would-be osteoclasts from blood; rather, its effects on migration would be local and serve to reposition responsive plaque macrophages around developing osteoblast-like cells and to promote their differentiation into vascular osteoclasts. Because deletion of the RANKL decoy receptor osteoprotegerin leads to enhanced calcification in atherosclerotic plaques,\(^14\) the findings of Byon et al support the concept that osteoclast-like cells within plaque may paradoxically accelerate rather than reduce plaque calcification by suggesting that RANKL does not have additional roles in osteoblast differentiation. Much remains to be delineated along these lines, because the mechanisms whereby putative bone resorptive cells promote growth of bone-like structures in plaque rather than keeping them in check is not completely clear. More research on the macrophages that develop into osteoclast-like cells in plaques is needed.

In addition to the intense research interest in vascular calcification, the heterogeneity of macrophages in plaques is a hot topic. However, these topics too rarely meet up. Although CD11c expression may mark dendritic cells in plaques\(^15,16\) or M2 macrophages,\(^17\) we have been impressed that plaque phagocytes staining most intensely for CD11c appear multinucleate and accumulate at the perimeter of necrotic areas\(^18\) that will likely go on to calcify over time. CD11c is also prominent on osteoclasts developed in vitro from granulocyte macrophage colony-stimulating factor–de-
rived bone marrow dendritic cells. Thus, the work of Byon et al emboldens us to study CD11c^{hi} plaque macrophages in more detail and suggests that macrophage biologists interested in atherosclerotic plaque should expand beyond the M1/M2 paradigm and routinely add osteoclasts to the list of plaque macrophage phenotypes studied.

Dissecting out the origins of vascular calcification is proving to be harder than it looks. Several generations after the seminal observations of Mönckeberg, we still seem a long way from an effective cure or primary treatment strategy for this disease. Yet, as evidenced by the work of Byon et al, piece by piece we seem to be slowly putting the puzzle together.

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Disclosures

None.

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Key Words: atherosclerosis ▪ calcification ▪ macrophages ▪ vascular biology ▪ vascular smooth muscle cells

Figure. Schematic diagram of pathways of vascular calcification. Oxidative stress induces Runx2 expression, with Runx2 then binding to the RANKL promoter and increasing RANKL expression in VSMCs, which serves to attract macrophages but is not required for the calcifying activity by VSMCs per se. These pathways orchestrate the vascular calcification process via VSMCs, macrophages, and their differentiated progeny. Cell schematics for macrophages, osteoclast-like cells, and calcifying vascular cells are reproduced from Kovacic et al.