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The leading edge of vascular calcification

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About two decades ago, when lipids were the focus of atherosclerosis research, calcific vasculopathy (or, more commonly, “vascular calcification”) had been dismissed as a non-biological, unregulated, degenerative phenomenon. Ironically, decades earlier, atherosclerosis itself had been dismissed in the same manner. In the earlier 1990s, a series of events raised awareness of calcific vasculopathy. New imaging modalities such as electron-beam and ECG-gated computed tomography showed coronary calcification to occur earlier and more often than previously believed, to the point that it is now used widely as a “pre-clinical” rather than end-stage marker of atherosclerosis and cardiovascular disease. Another event was the discovery of bone differentiation effectors and markers, such as bone morphogenetic protein, within human atherosclerotic plaque and vascular cells. This finding introduced a new paradigm, that vascular calcification is a regulated process similar to osteogenesis.¹ The first review on this new concept appeared in a 1994 issue of this journal.² Since then, the rate of publications on the topic has grown exponentially, from about ten articles per year to nearly eight hundred.

A review in this issue by Leopold³ addresses in detail much of what is now known about the form of vascular calcification that occurs in the arterial media, and shows great advances in the field. However, given that inspiration for further scientific advancement is triggered in the realm of what is not known, we have gathered here a few of the open and frequently asked questions and considerations that help define the edge of knowledge in this field:

Is calcific vasculopathy the body's adaptive response to a perceived threat? A mechanical adaptive value has been proposed. By theoretical analysis, mineral may reinforce plaque, by reducing certain components of stress in adjacent regions. However, theory also predicts that compliance mismatch of a rigid inclusion will increase other components of stress at the edges facing in the direction of the stress.⁴ This remains an unsettled area. Another possible adaptive value is immunological. Soft tissue often mineralizes in response to large or resistant opponents, such as helminths, abscesses, or foreign bodies. In these cases, calcification may serve as an ultimate immune defense that sequesters a recalcitrant foe

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inside a wall of bone. Thus, calcific vasculopathy may develop if the immune system considers atherosclerotic plaque in a similar vein.

What cells produce the mineral and where do they originate? In both intimal and medial calcification, as in bone, the mineral forms extracellularly. Cells release microvesicles and generate a permissive extracellular matrix, both of which provide nucleation sites for crystal formation.⁵ Most evidence suggests that the cell of origin is some type of mesenchymal from a resident progenitor subpopulation, from marrow-derived mesenchymal stem cells, or from vascular smooth muscle cells that have de-differentiated and re-differentiated or that have transdifferentiated into osteoblast-like cells. Recent reports also suggest a role for vascular endothelial cells either through endothelial-mesenchymal transformation into osteoblastic cells or as a source of calcifiable microvesicles carrying bone morphogenetic protein.^{6, 7}

Is there a coupling between osteoblastic and osteoclastic cells in the vasculature? One possible coupling factor is osteoprotegerin (OPG), which is produced by osteoblasts and blocks osteoclastic bone resorption by serving as a decoy receptor for a key osteoclast differentiation factor, receptor activator of NF- κ B ligand (RANKL). Mice deficient in OPG, which have more osteoclastic cells or activity in skeletal bone, were unexpectedly found to have vascular calcification.⁸

On one hand, the phenotype may result from direct, unopposed effects on vascular cells of RANKL, which also regulates apoptosis. On the other hand, in skeletal bone, osteoclasts are known to stimulate osteoblastic activity, a coupling that contributes to ongoing bone remodeling. A coupling between these cells in the vasculature would have important ramifications.⁹

What causes microvascular calcification? Also known as “calciphylaxis” and, more properly, calcific arteriolopathy, it appears to associate with renal disease.¹⁰ This serious condition can lead to tissue necrosis and gangrene, but studies of its mechanism and treatment remain at an early stage.

Do calcium (hydroxyapatite) crystals initiate inflammation? Evidence suggests that inflammation promotes calcification, but a positive feedback loop may also occur. Pyrophosphate crystals are known to drive inflammation in arthritic conditions such as pseudo-gout or calcium pyrophosphate deposition disease, and cholesterol crystals have been shown to induce inflammatory programs.¹¹ Evidence also suggests that calcium hydroxyapatite crystals are taken up by cells and directly affect inflammatory gene expression.¹²

Why is some cardiovascular calcification in the form of amorphous hydroxyapatite and some in the form of bone tissue? In some cases, the mineral develops into bone tissue, with its full architectural features.¹³ This occurs more often in intimal than in medial calcification. In embryogenesis, formation of bone tissue requires vascular invasion of amorphous mineralized matrix, where the vasculature provides the scaffold for the architecture. In vascular calcification, the bone-like tissue often blends with amorphous

mineral or appears to arise from it, a feature also found in embryonic ossification. Whether amorphous mineral is a precursor for bone tissue in the vasculature remains to be seen.

How does calcific vasculopathy lead to its clinical consequences? Many effects are thought to result from loss of vascular compliance and aortic recoil: systolic hypertension, diastolic hypotension, and left ventricular hypertrophy. The diastolic hypotension may, in turn, cause coronary ischemia and watershed myocardial infarction. Loss of aortic recoil (Windkessel effect) also increases demand for cardiac energy. This may account for some cases of diastolic dysfunction and congestive heart failure. In calcific aortic valve stenosis, the ventricle eventually fails to generate enough pressure to maintain its own diastolic coronary perfusion, ultimately leading to hemodynamic collapse.¹⁴ Another related mystery is why and how calcific aortic valve disease affects only the aortic face of the cusps. A role for fluid mechanical environment is likely, and differences in gene expression have been identified. Which, if either, is primary, remains undetermined.

Is regression possible? Since cardiovascular calcification occurs more often in individuals with osteoporosis, independently of age,¹⁵ drugs for osteoporosis have been tested for effects on calcific vasculopathy. One of these is the potent bone *anabolic* therapy for osteoporosis, parathyroid hormone injection. Seminal work from the Towler laboratory suggests it may prevent cardiovascular calcification in hyperlipidemic mice.¹⁶ Since the vast majority of patients receiving PTH are mature women, it may be possible to follow effects on vascular calcification by assessing breast arterial calcification in their routine mammograms. Such a study may answer the key question of whether such treatment has benefits in the context of preexisting atherosclerosis. *Anti-resorptive* treatments for osteoporosis, such as bisphosphonates, also appear to inhibit aortic calcification in animals. Unfortunately, this effect requires doses that also attenuate bone formation.¹⁷

Much open territory remains in the field of vascular calcification, but it is advancing to the point where efforts toward therapeutic interventions are ready to expand in earnest.

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