Osteopontin
Between a Rock and a Hard Plaque

Linda L. Demer, Yin Tintut

Vascular calcification is widely regarded as merely a rare, end-stage, passive, degenerative, and inevitable process of aging. Decades ago, atherosclerosis had been similarly dismissed, but extensive research finally demonstrated its active regulation. Current research is now revising outdated views of vascular calcification. New imaging techniques made it clear that coronary calcification is neither rare nor end stage but occurs in 90% of patients with coronary artery disease and that the vast majority of significant coronary stenoses are calcified. Coronary calcification is also associated with increased cardiovascular risk.

One clue to the regenerative—rather than degenerative—nature of vascular calcification is that it often includes histopathological features of bone. In the 1700s, Morgagni and others described arterial ossification in their postmortem examinations: “...the left coronary artery appeared to have been changed into a bony canal from its very origin.” In 1863, Virchow labeled the vascular changes as “ossification, not mere calcification, occurring by the same mechanism by which an osteophyte forms on the surface of bone.” In 1906, marrow was described within the bone tissue within the arteries—a richly vascularized cellular red marrow containing adipocytes, neutrophils, eosinophils, lymphoid and erythroid cells, reticulocytes, megakaryocytes, and other characteristic elements of marrow. Bunting further described (and we have also observed) evidence of resorption by osteoclast-like cells in this tissue. Within the vascular tree, the aorta and cardiac valves are the most common sites of ossification, and these contain all the stages of osteogenesis from the youngest variety of osteoid tissue up to true bone. Calcification of cardiac valves also involves lipid deposition and expression of bone matrix proteins. Because of various selection bias effects, the ossified form of vascular calcification may be more common than currently believed. One pathologist argued that the ossified lesion “is regarded as rare, chiefly because it is not more often searched for.”

The concept that vascular calcification is living tissue undergoing active remodeling suggests that it could be reversed through biological manipulations, in effect, producing osteoporosis locally in the artery wall. In this issue of Circulation Research, Wada et al. elucidate one of the mechanisms governing vascular calcification that may lead to a therapeutic approach. Using ultrastructural localization and dynamic measurement of mineral deposition, the authors provide strong evidence for the role of the bone matrix protein osteopontin in inhibition of vascular calcification. This dose-dependent inhibition was shown to be most likely due to direct association of osteopontin with growing apatite crystals.

Osteopontin (OPN), an acidic phosphorylated glycoprotein, was named for its function as a bridge between cells and mineral. Forming a proteaceous coating over the solid crystal surface, OPN mediates attachment of both osteoblasts and osteoclasts to bone mineral through interaction of its highly conserved GRGDS sequence with integrins. In cell-free solutions and gels, OPN inhibits apatite crystal formation. For this reason, OPN, which is present in urine and other body fluids, is believed to prevent stone formation.

Other bone matrix proteins also inhibit mineralization by blocking growth or delaying nucleation and some, such as bone sialoprotein, initiate crystal nucleation. Many of these are also expressed in the artery wall or in atherosclerotic lesions. Osteocalcin (bone gla protein) binds to apatite and is believed to inhibit crystal formation based on in vitro studies and a genetically modified mouse model. When gamma carboxylated at glutamic acid residues by a vitamin K-dependent carboxylase, osteocalcin binds avidly to bone mineral. A related protein, matrix gla protein (MGP), which contains 5 gamma-carboxyglutamic acid (gla) residues, is also believed to inhibit mineralization, based on a mouse model described later. Some uncertainty remains concerning the function of bone matrix proteins, in that their behavior in cell-free solution or in gel-agarose medium may differ from that in a complex, fibrillar intercellular matrix in which proteins may undergo function-altering configurational changes. The purpose of several proteins, all with the same function—binding to and inhibiting growth of hydroxyapatite mineral—is unclear. One possibility is that the different lattice geometries on the different faces of the mineral crystal have different binding avidity for the different matrix proteins. This geometric selectivity would permit independent control of growth along each of the crystal axes. Thus, genetic regulation of the relative abundance of the various matrix proteins would actually govern crystal shape.

Paradoxically, bone matrix proteins that inhibit apatite formation are found at increased levels in calcified human atherosclerotic plaque and in culture. One might expect that reduced levels of these factors would be necessary to permit mineralization. One possible explanation is that the inhibitory factors are induced in response to mineralization.
but that they are outstripped by the mineralization rate. Thus, their expression would be associated with mineralization but not causal.

It was an unexpected observation that stimulated reconsideration of the pathogenesis of vascular calcification. Giachelli et al. found that OPN expression distinguished pup versus adult smooth muscle cells in subtraction hybridization studies. Together with the prior description of matrix vesicles in calcified human atherosclerotic lesions, the possibility arose that the bone-like nature of vascular calcification could be demonstrated at the ultrastructural and molecular levels. Our group hypothesized that vascular calcification recapitulates embryonic osteogenesis at the molecular level and demonstrated expression of the embryonic differentiation factor, bone morphogenetic protein (BMP)-2, in calcified carotid plaques and in an in vitro model of vascular calcification. A stream of reports followed showing bone extracellular matrix proteins in atherosclerotic lesions. Further evidence for genetic regulation came from the unexpected demonstration of pervasive endochondral ossification of the aorta and its branches in the matrix gla protein knockout mouse.

To study the cells in the artery wall that are responsible for producing mineralized matrix, our group isolated from cultures of bovine aortic smooth muscle cells (BASMCs) a minority of clones, termed calcifying vascular cells, that underwent osteoblastic differentiation and mineralization in vitro. Consistent with this, Wada et al. found marked heterogeneity in the bone differentiation marker, alkaline phosphatase activity, in their BASMC cultures. This contributes further to the already overwhelming evidence for smooth muscle cell heterogeneity. These findings underscore the importance of accounting for the mix of cellular subtypes in studies of smooth muscle cells from the artery wall by immunological characterization or cloning.

Although the mineral produced in culture is the bone mineral hydroxyapatite, it lacks the characteristic features of bone tissue; that is explained by the absence of vascular ingrowth, which is required for fully formed tissue. As would be expected, in atherosclerotic lesions, it is generally accepted that most calcium deposits also lack tissue organization and vascularization. However, absence of full tissue organization at one stage does not exclude a role of osteogenic processes. In the embryo, both types of osteogenesis, intramembranous and endochondral, are preceded by a phase of amorphous crystallization in a cell-free matrix, such as calcified cartilage. Bone structures, including osteons, Haversian canals, lacunae, and canaliculi form later, only after angiogenic invasion. Indeed, bone structures are found in the artery wall only where vessels interface with calcium deposits. These observations strongly suggest that the nonbone form represents an early stage of ossification.

Whether arterial ossification is common is not the key issue. That it occurs at all is extraordinary. Such transformation of tissue raises questions about cell identity determination, terminal differentiation, and the possibility of pluripotent mesenchymal stem cells in vascular tissue. If arterial cells can form bone, can arteries and other connective tissues be used to produce replacement tissue? The hypothesis that embryonic connective tissue stem cells remain in adult tissues is gaining acceptance, particularly with regard to the marrow stroma. Indeed, our “calcifying vascular cells” may represent mesenchymal progenitor cells with pluripotentiality one hierarchical level below that of marrow stromal cells on the basis of their apparent lack of adipogenic potential.

The findings of Wada et al. may lead to consideration of OPN as a soluble inhibitor of mineralization as a long-term possibility. Yet, other functions of osteopontin must not be forgotten. On the basis of findings in the OPN knockout mouse, its role in wound healing may go beyond its role in bone formation. OPN is even regarded as the culprit in other disease contexts, such as hepatic fibrosis, for which OPN inhibitors may be considered potential therapeutic agents.

References


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