C alcification of the arterial wall occurs in, among others, atherosclerosis and aging. The role of calcification in plaque stability is subject of ongoing discussion; media calcification in aging and diabetes increases stiffness and pulse pressure. It is now clear that calcification is not simply a passive process but, rather, is tightly regulated, involving induction of an osteochondrogenic phenotype in the vascular smooth muscle cells (VSMCs) and control of expression and activity of factors promoting and inhibiting calcium deposition. In this issue of Circulation Research, Johnson et al\(^1\) provide evidence that transglutaminases (Tgases), cross-linking enzymes with recently discovered vascular functions, are required for inducing the vascular calcification process.

Tgases are multifunctional enzymes acting in the cell, at the cell surface, or as released enzymes. Their most prominent feature is the covalent cross-linking of proteins.\(^2\) The glutamine–lysine cross-links, either within or between proteins, result in the stabilization of extracellular matrices and the binding of signaling molecules, such as osteopontin, to the matrix but also the prolonged activation of angiotensin II receptors through dimerization.\(^3\) Cell surface Tgases are involved in cell adhesion through integrin clustering and function as coreceptors in the binding to fibronectin.\(^4\) The Tgase family consists of 9 members, of which TG1, TG2, and the coagulation factor XIII are expressed in the arterial wall.\(^5\) The tissue-type Tgase studied here (TG2) fulfills both cross-linking activities and G protein functions. Mice deficient in TG2 have no obvious cardiovascular phenotype. However, when challenged, arteries show impaired capacity to undergo inward remodeling in response to reduced blood flow\(^6\) and hypertension.\(^7\)

Johnson et al\(^8\) studied the role of TG2 in the calcification program of cultured VSMCs and aortic rings in organ culture. TG2 is indeed involved in orthotopic bone formation. The present study follows previous work of the authors\(^9\) on the fundamental role of TG2 in chondrocyte differentiation during growth plate mineralization and pathological cartilage calcification. Central to their present work is the switch of VSMCs from a contractile to an osteochondrogenic phenotype. It has long been known that VSMCs may lose their contractile phenotype and switch to a synthetic phenotype in culture\(^10\) and during vascular injury and repair.\(^11\) In cell cultures, such switches depend critically on the substrate composition. Yet, a broad class of “synthetic” phenotypes exists, the regulation of which is subject of ongoing work.

The Figure summarizes the sequence of events leading to calcification in the study by Johnson et al. Factors known to induce the osteochondrogenic phenotype are bone morphogenetic protein-2,\(^12\), a member of the transforming growth factor-\(\beta\) superfamily, and inorganic phosphate (Pi).\(^13\) Bone morphogenetic protein-2 is produced by several cell types in atherosclerotic lesions, including endothelial cells, foam cells, and VSMCs,\(^1\) whereas increased Pi is a hallmark of chronic kidney disease. Johnson et al studied the effects of these two stimuli, with an emphasis on Pi, on the phenotype and function of wild-type and TG2 knockout (KO) cells. In 1 of the models, cells were removed from laminin to induce the loss of the contractile phenotype. Such loss is characteristic of the changes seen in vascular injury and repair. Simultaneously, an osteochondrogenic phenotype was induced by high Pi. Under these conditions, the wild-type cells showed upregulation of calcification promoters and formation of calcified nodules, demonstrating induction of the calcification program. The KO cells switched from contractile to a noncalcifying phenotype, characterized by upregulation of inhibitors for calcification. It is not fully clear how this synthetic phenotype should be classified. As expected from the pattern of gene expression, calcified nodules remained absent. Extrinsic Tgases (including factor XIII) could rescue this calcification process in the KO, provided that the catalytic site was present but not requiring the GTP-binding site. Also, pretreatment of matrix with TG2, through retention of this enzyme, could direct the VSMCs toward calcification. The involvement of TG2 in the calcification process was further demonstrated by short hairpin RNA knockdown and extrinsic TG2 in human VSMCs. These results were repeated in mouse aortic rings in culture, where calcification following Pi stimulation was absent in the KO vessels and could be blocked by a TG2 inhibitor in wild-type vessels. Together, these experiments convincingly demonstrate that TG2 activity is required for calcification. The results obtained on the cultured vessels, where initial dedifferentiation is assumedly less than in the cell cultures, underline the role of TG2 in calcification in a normal matrix environment.

What is the mechanism? Unraveling the signaling downstream of TG2 activity was not the main purpose of this study and clearly needs to be addressed in future work. One could...
argue that lack of TG2 simply maintains the VSMCs in a contractile state, preventing the calcification program. However, the reverse was true: TG2 expression was actually required for maintenance of a contractile phenotype of VSMCs grown on laminin. The effects of extrinsic Tgases and matrix pretreatment with TG2 point at an extracellular, catalytic site-dependent effect. Possibilities include a change of the mechanical properties of the matrix, incorporation of signaling proteins, and facilitation of cell-matrix interactions at focal adhesion sites.

Although some evidence exists,16 it remains to be firmly established whether the present findings on cell and vessel cultures translate into a relationship between local Tgase activity and calcification during atherosclerosis and aging. Cross-breeding murine models for calcification with the TG2 KO mouse and immunohistochemical analysis of human tissue will be the first step to better define the regulation and mechanisms of action of these enigmatic but fascinating enzymes in the vascular wall.

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None.

**References**


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