Calcification 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 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. 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. Cell surface Tgases are involved in cell adhesion through integrin clustering and function as coreceptors in the binding to fibronectin. The Tgase family consists of 9 members, of which TG1, TG2, and TG6 are required for inducing the vascular calcification process. Also, pretreatment of matrix with TG2, through retention of the catalytic site was present but not requiring the GTP-binding site. Absent. Extrinsic Tgases (including factor XIII) could rescue calcification in the KO, provided that the cation is required for calcification. The results obtained on 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 assuming 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 cultures translate into a relationship between local Tgase activity and calcification during atherosclerosis and aging. Considering the widespread role of the Tgases, it may be more realistic to aim at influencing local regulation or downstream targets than at direct inhibition. In any case, the first step will be to better define the regulation and mechanisms of action of these enigmatic but fascinating enzymes in the vascular wall.

**Sources of Funding**

E.B. is funded by the Netherlands Heart Foundation (grant 2001D038).

**Disclosures**

None.

**References**


Key Words: transglutaminase | atherosclerosis | smooth muscle cell | calcification
A Vascular Bone Collector: Arterial Calcification Requires Tissue-Type Transglutaminase
Ed VanBavel and Erik N.T.P. Bakker

doi: 10.1161/CIRCRESAHA.108.173013
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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