Vascular Calcification
It’s All the RAGE!
Dwight A. Towler

Macrovacular calcification increasingly afflicts our aging and dysmetabolic population. Once considered only a passive process of dead and dying cells, data from multiple laboratories worldwide have converged to reveal that vascular calcification is in great part an actively regulated form of matrix mineral metabolism. A uniquely horrendous situation arises in end-stage renal disease. Antecedent vasculopathy from diabetes, dyslipidemia, or hypertension interacts with dialysis-modulated uremia—a fluctuating hyperphosphatemic and hyperphosphatemic milieu that increases vascular smooth muscle cell (VSMC) apoptosis, overwhelms defenses against soft tissue mineralization, and promotes low-grade panvascular inflammation. Elegant genetic studies by Cecil and Terkeltaub,
 coupled with the enlightening work of Festing et al and Li et al have highlighted the important role of pyrophosphate and phosphate metabolism in the pathobiology of arterial calcification. In addition, oxidative stress signals (reactive oxygen species [ROS]) elaborated in response to key inflammatory cytokines—namely, interleukin 6, interleukin 1, and tumor necrosis factor—have been shown to participate in vascular activation of the osteochondrogenic gene program characteristic of bone formation. Only recently, however, has signaling via the receptor for advanced glycosylation end products (RAGE) been implicated as a critical contributor to both ROS-regulated and pyrophosphate-regulated vascular calcification. RAGE is an immunoglobulin superfamily member, initially identified by Yan et al as an endothelial cell surface receptor for glycated proteins that accumulate with hyperglycemia. Although membrane-bound RAGE promotes nuclear factor-kB and ROS signaling, soluble RAGE (sRAGE) functions as a dominant-negative “faux receptor” for RAGE-activating ligands (Figure). Indeed, sRAGE levels are reduced in patients with calcific aortic stenosis, suggesting that unchecked RAGE-dependent inflammation may contribute to valvular calcium load. As immediately germane to the pathobiology of diabetic arterial calcification, carboxymethyl lysine and other advanced glycation end-products bind RAGE and sRAGE as ligands. However, RAGE functions as a crucial signal transducer for HMGBl and S100A/calgranulin family members, proteins released with cell necrosis and leukocyte activation, respectively. Seminal studies from Hofmann Bowman et al first identified expression of S100A12—a human RAGE ligand—in aortic aneurysms. Furthermore, they showed that transgenic expression of human S100A12 in VSMCs promotes dilating aortic matrix remodeling in mice. Although elastinolysis and oxidative stress—stimuli for vascular calcification—were concomitantly upregulated by the S100A12 transgene, the impact on arteriosclerotic calcium accrual was not previously addressed.

In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Hofmann Bowman et al directly examine the role and regulation of the S100A12/RAGE axis in vascular calcification. Implementing the apolipoprotein E–/– murine model, they show that selective VSMC expression of S100A12 increased medial calcium accrual by 2- to 6-fold in proximal aorta and innominate arteries, respectively. Concomitant increases in bone morphogenetic protein-2 and Runx2—master regulators of osteogenic mineralization—were also elicited by the S100A12 transgene. Robust responses were observed in apolipoprotein E–null mice, a permissive background for time-dependent medial osteochondral metaplasia even on standard rodent chow diets. Ex vivo, S100A12 upregulated osteogenic gene expression and mineralization of cultured transgenic VSMCs. Interestingly, the proosteogenic propensity elicited by the S100A12 transgene required conditioned media from lipid-challenged apolipoprotein E–null macrophages; this presumably reflects contributions of oxysterols, tumor necrosis factor, or other signals elaborated by the monocyctic/macrophage lineage that augment the osteogenic milieu.

Why is this study so enlightening? There are several important reasons. First, it provides compelling, independent yet convergent evidence for the crucial role of oxidative stress and NAD(P)H oxidase signaling in arterial calcification. Intriguingly, downregulation of VSMC Nox1 has also been implicated in the inhibition of medial calcification in other settings. Second, RAGE ligands, such as S100/calgranulins, are not normally expressed in VSMCs in the absence of injury. Thus, the capacity of a uniquely human RAGE agonist, S100A12, to promote VSMC osteochondrogenic mineralization in transgenic mice provides strong evidence that the paracrine S100A12/RAGE axis enhances vas-
cular calcium accrual. Apolipoprotein E deficiency likely affords the elaboration of macrophage-derived humoral signals that synergize with S100A12, as well as osteogenic morphogens, to drive arteriosclerotic medial calcification (Figure). Third, the report introduces a new view of the mechanisms whereby S100/calgranulins upregulate ROS production by VSMCs—namely, via direct cell surface Nox1 activation (Figure). Hoffman Bowman et al demonstrate protein-protein interactions between Nox1 and S100A12, and similar interactions may occur with other Nox members in other contexts. Given the prior evidence that RAGE agonists, such as S100/calgranulin, increase ROS production, a heterodimeric RAGE-Nox1 signaling complex may mediate ROS generation and osteogenic mineralization in VSMCs (Figure). This model is supported by data demonstrating that sRAGE inhibits S100A12-induced osteogenic gene expression and calcium deposition in cultured VSMCs. Finally, when taken together with very recent data from Cecil and Terkeltaub—data demonstrating that RAGE conveys osteogenic arterial calcification signals activated by pyrophosphate deficiency—the work of Hofmann Bowman et al provides evidence that the RAGE ligand S100A12 binds both RAGE and Nox1, induction of the putative Nox1-RAGE heterodimeric complex by S100A12 has yet to be directly demonstrated. Whether other RAGE ligands also interact with Nox1 is unknown.

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References

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