Matrix Remodeling in Vascular Calcification Associated with Chronic Kidney Disease

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ABSTRACT

Vascular calcification is a major contributor to cardiovascular disease, a leading cause of death in patients with chronic kidney disease. Mechanistic studies highlight the importance of dysregulated mineral metabolism, vascular osteochondrogenic processes, apoptosis, and deficiencies in calcification inhibitors as potential mediators of calcification in renal disease. However, the contribution of the extracellular matrix in vascular calcification associated with chronic kidney disease is less understood. Here we examine evidence that suggests important roles for elastin and elastin-degrading enzymes as potential key regulators of calcification. Additional studies aimed at further understanding their role are critical for the design of therapeutic interventions.

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Vascular calcification, the inappropriate deposition of calcium-phosphate mineral, is typically observed in blood vessels, myocardium, and cardiac valves. Vascular calcification is prevalent in normal aging, chronic kidney disease (CKD), and cardiovascular diseases such as atherosclerosis. On the basis of the location of the apatite, vascular calcification can be divided into two distinct types: an intimal form associated with atherosclerosis concurrent with inflammation, lipid deposition, and development of occluding plaques and lesions, and a medial form also known as arterial medial calcification, Mönckeberg's sclerosis, or elastocalcinosis that occurs along and between the elastic lamellae of the arterial medial layer.[1]

The incidence of arterial medial calcification strongly correlates with cardiovascular events and is a strong prognostic marker of mortality in patients with ESRD.[2,3] Arterial medial calcification in large blood vessels leads to increased stiffness, pulse wave velocity, and pulse pressure, resulting in significant mechanical changes in the arterial wall that alters distensibility.[2] Pronounced increase in vascular stiffness also leads to hypertension, left ventricular hypertrophy, heart failure, and compromised coronary perfusion.[3]

Several mechanisms have been proposed for the development of vascular calcification in CKD:[1] (*1*) dysregulated mineral metabolism clearly correlates with vascular calcification and mortality in CKD patients;[1,4] (*2*) strong evidence obtained from experimental models of kidney disease, as well as human patients, indicates that osteochondrogenic processes contribute to vascular calcification in CKD;[5,6] (*3*) deficiencies in calcification inhibitors, such as fetuin-A and pyrophosphate, are linked to vascular calcification in dialysis patients and correlate with increased mortality;[5,7] and (*4*) cell death is a major regulator of vascular calcification in CKD patients[8] and matrix vesicles released from damaged or dead vascular smooth muscle cells (VSMCs) create a focal point for the initiation of calcification.[5] Finally, recent studies implicate elastin and elastinolysis as mediators of vascular calcification in CKD, and are thus the main focus of the present discussion.

ROLE OF ELASTIN IN VASCULAR CALCIFICATION

Elastin, a key constituent of the extracellular matrix in elastic arteries, is secreted from VSMCs as a soluble monomer called tropoelastin. Tropoelastin interacts with fibrillin or microfibril-associated glycoprotein and is oriented into proper alignment for cross-linking by lysyl oxidase. This cross-linked structure provides elastin with extensive tensile strength critical for the contractile function of VSMCs and hemodynamic properties of the vessel. The calcium-binding capacity of elastin was initially observed in the early 1970s, where it was proposed that the positively charged calcium ions attract phosphate ions, thereby facilitating apatite nucleation and subsequent calcification.[9] Thus, calcification of elastin leads to increased stiffness that can

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ultimately lead to loss of vessel compliance.[2]

ELASTIN DEGRADATION AND MATRIX METALLOPROTEINASES IN VASCULAR CALCIFICATION

Elastin degradation is also important for the initiation and progression of vascular calcification. Matrix metalloproteases (MMPs) are implicated in vascular calcification. Members of the MMP family, including gelatinases (metalloelastases and matrilysins), degrade insoluble elastin. Tissue inhibitors of MMPs regulate MMP activity by providing a feedback mechanism to prevent excessive matrix degradation. In disease conditions, an imbalance between MMPs and tissue inhibitors of MMPs could cause excessive MMP activity and may lead to pathologic changes in the vessel wall.[10]

Simionescu *et al.* first observed the association between elastin calcification and increased MMP expression.[11] They demonstrated that subdermally implanted glutaraldehyde-treated bovine parietal pericardium expresses an array of matrix proteinases including serine proteinases and MMPs. Later, Vyavahare *et al.* identified the overexpression of MMP-2 and -9 co-localizes with calcifying elastin fragments in subcutaneous purified elastin implants. Not surprisingly, local delivery of synthetic MMP inhibitors significantly mitigates this elastocalcinosis.[12] Aluminum chloride pretreatment of elastin also leads to inhibition of MMP-mediated elastocalcinosis in a subdermal implantation model as well as in mitral valve replacement studies[13] since aluminum binds irreversibly to elastin, altering the spatial structure and rendering it resistant to MMP cleavage and calcification. Basalyga *et al.* also demonstrated that periadventitial treatment of abdominal aortas with low concentrations of calcium chloride induces chronic degeneration and calcification of elastic fibers. This occurs in the absence of aneurysm formation and inflammation, which is phenotypically similar to arterial medial calcification. Consistent with the importance of elastin degradation in vascular calcification, aortas from MMP-2 and -9 single null mice do not calcify, presumably because MMP-9 is not active to degrade the elastin.[14]

More recently, Qin *et al.* observed that aortic calcification in two different arterial medial calcification models is significantly reduced compared with untreated controls after systemic treatment with the MMP inhibitors, doxycycline and GM6001.[15] Likewise, Bouvet *et al.* examined the role of MMPs in medial elastocalcinosis using warfarin/vitamin K treatment in rats, an experimental model of matrix-gla protein deficiency. MMP-9 activity and TGF-β signaling increase early and before calcification, and blocking MMP activation with doxycycline or TGF-β signaling with SB-431542 mitigates calcification.[16]

The mechanism by which degraded elastin promotes vascular calcification is not certain but at least two possibilities exist. First, elastinolysis increases elastin affinity for calcium binding thereby facilitating epitactic growth of hydroxyapatite along the elastic lamellae.[17] Second, elastinolysis induces the release of soluble elastin peptides and TGF-β that interacts with elastin-laminin receptor and TGF-β receptor, respectively.[18] It has also been shown that TGF-β stimulates bovine aortic medial cells to calcify in culture.[19] Although the exact mechanism responsible for this effect is not yet known, several pieces of data support the idea that elastinolysis directly affects the phenotype of VSMCs, thereby regulating their potential to direct matrix calcification. Simionescu *et al.* demonstrated that aortic VSMCs incubated with elastin peptides exhibit an increased expression of elastin-laminin receptor, MMP-2, and bone-related proteins, including Runx2/Cbfa1, osteocalcin, and alkaline phosphatase.[18] Expression of osteogenic genes in VSMCs is further enhanced by the addition of TGF-β along with the elastin peptides, even in the absence of any other mineralizing agent. More recently, elastinolysis is implicated directly in phosphate-induced VSMC calcification *in vitro*. VSMCs cultured with high phosphate show significantly accelerated calcification upon treatment with α-elastin, a degradation product of elastin. Furthermore, expression of osteoblast differentiation markers significantly increases in the presence of α-elastin. No calcification was observed with α-elastin under normal phosphate conditions.[20] These *in vitro* data indicate that pathologic degradation of elastin leading to generation of elastin peptides may either initiate or accelerate calcification by inducing a phenotypic change in VSMCs.

ELASTIN DEGRADATION AND VASCULAR CALCIFICATION IN CKD

Is elastin degradation a potential mechanism contributing to vascular calcification in CKD? Although very few studies have examined artery wall elastin in experimental models of uremia, Amann *et al.* notes that subtotally nephrectomized rats display decreased relative content and focal rupture of elastin fibers compared with sham-operated rats, although these changes occurred in the absence of calcification.[21] Similarly, our group has shown that elastin turnover, as measured by desmosine content and histochemical staining, elevates in uremic, high-phosphate–fed mice and precedes arterial medial calcification. In these studies, levels of both MMP-2 and MMP-9 elevate with time in the calcified artery (unpublished findings). Finally, Aikawa *et al.* investigated the role of cathepsin-S, a major macrophage elastase, in atherosclerotic calcification in uremic mice. Uremic, cathepsin-S–deficient $ApoE^{-/-}$ mice show significantly less arterial and aortic valve calcification as compared with controls. Cathepsin-S expression co-localizes with calcifying cells and fragmented elastin in the atheroma and inflamed aortic valves. Furthermore, human VSMCs treated with cathepsin-S fragmented elastin undergo osteogenic changes, a process augmented in phosphate-enriched culture medium.[22]

In humans, Ibels *et al.* observed disruption and reduplication of the internal elastic lamina in autopsy specimens of elastic arteries from uremic patients.[23] Similarly,

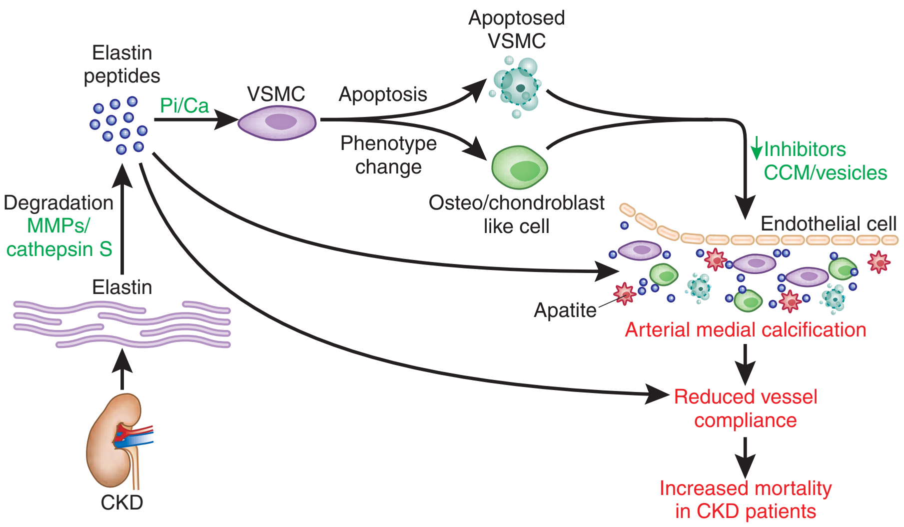

Figure 1. Uremia-driven elastinolysis may lead directly to reduced vascular compliance or indirectly to accelerated calcification by its degradation. Transient alterations in serum phosphate and calcium in CKD may be responsible for initiation of phenotype change of VSMC toward an osteochondrogenic lineage. Elastin degradation may accelerate this process. A cohort of osteochondrogenic and apoptotic VSMCs may contribute to calcium deposition by elaborating a calcification competent matrix, decreasing expression of calcification inhibitors, and/or releasing calcium phosphate–loaded vesicles. CCM, calcification competent matrix.

thinning and fragmentation of medial elastic fibers are present in epigastric arteries of dialysis patients undergoing renal transplantation, producing a strong correlation between MMP-2 upregulation and elastic fiber disorganization, stiffness, calcification, and vasomotor dysfunction.[24] Chung *et al.* also showed that diabetic arteries of a different set of patients with CKD demonstrated increased MMP-2 and MMP-9 activities by 42 and 116%, respectively, compared with nondiabetic arteries of patients with CKD. This enhanced MMP expression is highly correlated with arterial stiffness and pulse wave velocity.[25] Recently, Peiskerova *et al.* report that serum MMP-2 levels are higher in 80 patients with CKD stages 1 to 5 and 44 healthy control subjects.[26]

CONCLUSIONS

There is growing evidence for the importance of matrix remodeling in the initiation and progression of vascular calcification. However, our understanding of the matrix effects in arterial medial calcification associated with CKD is nascent. On the basis of the existing literature, a paradigm can be envisioned for the potential role of elastin, elastases, and matrix remodeling in arterial medial calcification associated with CKD (Figure 1). Clearly, future studies aimed at testing key components of this model are required to understand the importance of matrix remodeling in arterial medial calcification and potential mechanisms for its regulation.

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DISCLOSURES

None.

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