The Pathogenesis of Vascular Calcification in the Chronic Kidney Disease Mineral Bone Disorder: The Links Between Bone and the Vasculature

Keith A. Hruska, MD,[*,†] Suresh Mathew, MD,*[*] Richard J. Lund, MD,[‡] Imran Memon, MD,*[*] and Georges Saab, MD[†]*

Summary: Considerable scientific progress in the pathogenesis of vascular calcification that has accrued in recent years is reviewed in this article. Factors regulating mesenchymal cell differentiation and their role in the neointimal calcification of atherosclerosis and the vascular media calcification observed in chronic kidney disease and diabetes are discussed, as is the role of bone regulatory proteins in bone mineralization and vascular calcification. This includes recent studies related to fetuin-A, and the discovery of a new circulating hormone involved in regulating phosphate homeostasis and sensing skeletal hydroxyapatite precipitation. Finally, the relationship between skeletal mineralization and vascular mineralization is discussed in terms of their links, especially through serum phosphate concentrations.
Semin Nephrol 29:156-165 © 2009 Published by Elsevier Inc.
Keywords: *Vascular calcifications, matrix Gla protein, fetuin-A, phosphorus, cardiovascular disease, vitamin D, vascular smooth muscle*

Vascular calcification is a process that has been shown during the progression of atherosclerosis with neointimal calcification or with calcification of the tunica media. Although intimal and medial calcification have similarities, it is not clear that they follow a common pathogenesis, and both are stimulated by chronic kidney disease (CKD). Primary medial calcification, or Mönckeberg's sclerosis (MS), has been associated with CKD,[1] aging,[2] and diabetes.[3] In CKD, MS previously was thought to be a passive process,[1] occurring as a direct consequence of an increased calcium × phosphorus product. However, recent evidence discussed herein suggests that this is not the case. Rather, vascular calcification appears to be an active process involving a phenotypic drift towards an osteoblast-like cell secreting and mineralizing an extracellular matrix.

Understanding the pathogenesis of vascular calcification is essential because it is a frequent cause of morbidity and mortality for patients with CKD.[4] Indeed, vascular calcification occurs in all ages and stages of CKD.[5,6] However, the extent of coronary calcification appears to be more pronounced with a longer time on dialysis, older age, male sex, white race, diabetes, and increased serum calcium and phosphorous.[5] Coronary calcification is especially prone to be atherosclerotic and this form of vascular calcification has been shown to be due to differentiation of neointimal cells to an osteoblastic phenotype mineralizing their extracellular matrix akin to bone formation.

DIFFERENTIATION OF MESENCHYMAL STEM CELLS

Osteoblasts, smooth muscle myocytes, adipocytes, fibroblasts, and chondrocytes all share a

*Department of Pediatrics, Division of Nephrology, Washington University School of Medicine, St. Louis, MO.
†Department of Internal Medicine, Division of Nephrology, Washington University School of Medicine, St. Louis, MO.
‡Renal Division, Department of Medicine, Creighton University, Omaha, NE.
Supported by National Institutes of Health grants (DK070790, AR41677, and T32-DK062705 to K.A.H.), and by research support from Shire, Wayne, PA; Genzyme, Cambridge, MA; Abbott, Abbott Park, IL; and Fresenius, Waltham, MA.
Address reprint requests to Keith A. Hruska, MD, Department of Pediatrics, Washington University School of Medicine, Campus Box 8208, 5th Floor MPRB, 660 S. Euclid Ave, St. Louis, MO 63110. E-mail: Hruska_k@kids.wustl.edu
0270-9295/09/$ - see front matter
 doi:10.1016/j.semnephrol.2009.01.008

common mesenchymal progenitor stem cell. Differentiation along the osteogenic lineage requires crucial factors including the bone morphogenetic proteins (BMPs)[7] and Wnts, an amalgam of wingless (Wg) and int (Wnts).[8] The BMPs are part of the transforming growth factor-β superfamily that initiate signal transduction by binding to specific type II receptors, activating type I receptors, and affecting gene transcription through phosphorylation of regulatory Smad transcription factors. BMP-induced osteogenesis requires lineage- specific transcription factors such as *Runx2*, *Osx*, and *Msx1/2* that are key and include all of the proteins involved in osteoblast-mediated bone formation. Mice genetically engineered to be deficient in *Runx2* or *Osx* show a complete lack of ossification,[9,10] whereas those deficient in *Msx 1/2* have significant skeletal abnormalities.[11,12]

Thus, the process of osteogenic development and bone formation is critically and tightly regulated by the coordinated effects of the growth factors and transcription factors described earlier. Furthermore, the subsequent finding of these transcription factors expressed in calcified vessel walls has led to the theory that vascular calcification is also a tightly regulated, coordinated, and osteoblastic process. For example, low-density lipoprotein (LDL)–receptor negative (LDLR -/-) mice fed a high-fat diet developed vascular calcification associated with the expression of *Msx1* and *Msx2*.[13] In human beings, expression of *Msx2* and *Runx2* along with the chondrogenic transcription factor *Sox9* has been described in calcified atherosclerotic samples.[14] In addition, expression of the target genes of these factors such as alkaline phosphatase, osteocalcin, bone sialoprotein, and type II collagen also is increased. Other bone regulatory proteins such as matrix Gla protein (MGP) and osteoprotegerin (OPG) (both discussed later) are downregulated in calcified vessels compared with noncalcified vessels.[14] Deposition of bone matrix proteins also has been described in medial calcification associated with CKD.[15] These findings suggest that vascular calcification is an active process that simulates osteogenesis and bone formation.

THE VASCULAR SMOOTH MUSCLE CELL

Vascular smooth muscle cells (VSMCs) normally reside in the vessel wall media in a differentiated state wherein their contractile properties regulate vascular tone. However, the VSMC phenotype is characterized by the ability to reversibly enter a synthetic state of proliferation and production of large amounts of extracellular matrix.[16] The stimulus to change phenotype includes injury, various cytokines, growth factors, and certain components of the extracellular matrix. In the heightened synthetic state, VSMCs show decreased expression of contractile and adhesion proteins and a concomitant increase in cytoskeletal proteins.[17] In addition to various growth factors, culture of VSMCs in medium supplemented with serum has been shown to experimentally stimulate the transition to the synthetic phenotype.[18] Transition into the synthetic state is thought to be involved in the pathogenesis of atherosclerosis and MS.

After stimulation with serum, proliferating VSMCs grow to form a confluent monolayer.[19] Subsequently, areas of the monolayer develop multicellular foci that form nodular aggregates consisting of nonproliferating, quiescent VSMCs. Cells from these nodules appear to re-express markers of smooth muscle cell differentiation. Proliferating osteoblasts also form condensing nodules in culture.[20] Subpopulations of human and bovine aortic VSMCs have been shown to form these nodules and then calcify spontaneously.[21]

The addition of β-glycerophosphate, a phosphate donor, or high concentrations of inorganic phosphate have been shown to induce calcification in VSMCs in vitro,[22] and VSMCs from atherosclerotic donors showed increased expression of *Runx2*, osteopontin (OPN), and alkaline phosphatase.[23] Inorganic phosphate induced the expression of osterix and bone matrix proteins.[23] This action is stimulated through a sodium-phosphorus cotransporter in cultured VSMCs.[24,25] Thus, it appears that inorganic phosphate induces VSMCs toward an osteogenic phenotype in vitro. However, uremic serum also induces VSMCs to calcify, a process that is related partly to a factor that is independent of serum phosphate.