Inorganic Pyrophosphate: A Paracrine Regulator of Vascular Calcification and Smooth Muscle Phenotype

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Cellular and molecular similarities between orthotopic versus heterotopic mineral deposition are beginning to emerge. During bone formation, the major orthotopic venue, two general mechanisms drive tissue mineralization1 (Table). With endochondral ossification, an avascular cartilaginous skeletal template is first established by chondrocytes; this cartilage template calcifies and is subsequently resorbed and replaced by bone through osteoblast-mediated synthetic activity.1 With intramembranous ossification, osteoblast-mediated bone formation occurs directly in a type I collagen-based extracellular matrix, without preceding cartilage template formation.1 During vascular calcification, the major heterotopic venue, ossification mechanisms similar to those mediating bone formation contribute to vascular calcium load.2,3 At least 4 distinct histoanatomic variants of vascular calcification can be readily identified2,3 (Table). Eccentric lumen-deforming atherosclerotic calcification is associated with both osteogenic and chondrogenic gene regulatory programs in areas overlapping and adjacent to calcifying necrotic fibro-fatty plaques.4 With evolution to advanced disease, endochondral bone formation is observed.5-5 Medial arterial calcification, by contrast, is a concentric mural calcification process reminiscent of matrix vesicle-mediated intramembranous bone formation (Table).2,3 In cardiac valve calcification, valve thickening, stippled calcification, and degenerative fatty expansion in the valvular fibrous occurs early on; this is associated with mononuclear and T-cell infiltration and the elaboration of osteogenic gene expression.7 Finally, in the setting of an elevated calcium phosphate product, vascular tissue calciphiaxis occurs in concert with widespread soft tissue calcification, without requisite recruitment of active osteogenic or chondrogenic processes.8 Once considered benign, the deleterious clinical consequences of vascular calcification have now become clear.9,10

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In addition to more common acquired disorders (Table), several enlightening congenital disorders of arterial calcification have now been described in detail, including infantile “idiopathic” arterial calcification (IIAC; OMIM #208000).11,12 In IIAC, deficiency in ectonucleotide pyrophosphatase/phosphodiesterase I (NPP1) causes widespread vascular calcification, periarthritic calcium deposition, stenosis of medium and large muscular arteries, and early cardiovascular demise.11,12 Human with IIAC are afflicted with a type of medial artery calcification that is oriented along the internal elastic lamina and associated with fibrous intimal hyperplasia;11,12 this stenosing hyperplasia causes hypertension and cardiomyopathy.11,13 A series of very elegant studies by Terkeltaub, Millan, and colleagues have recently shown that NPP1 and another ectoenzyme, tissue nonspecific alkaline phosphatase (TNAP), tightly regulate tissue levels of pyrophosphate (PPI), a key modulator of tissue mineralization (Figure).14,15 Both PPI-regulating ectoenzymes are present on mineralizing matrix vesicles and the plasma membranes of calcifying cells.15,16 Previous views of PPI emphasized only the biophysical chelator-like role in preventing nucleation and propagation of tissue calcium deposition.14 However, in an important manuscript published in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology,17 the group extends their seminal observations15,16 by demonstrating that PPI also functions to stabilize the aortic vascular smooth muscle cell (VSMC) phenotype; PPI inhibits cartilaginous metaplasia of VSMCs, ie, the “trans-differentiation” of these cells into chondrocytes.2 They show that NPP1−/− mice exhibit medial artery17 as well as ligamentous15 calcification. Using RT-polymerase chain reaction (PCR), the authors demonstrate upregulation of Sox9 and Runx2/Cbfa1, two skeletal transcription factors necessary for endochondral bone formation1 in aortas of NPP1−/−/H11001/H11002 animals.17 Type II collagen and osteocalcin (a bone Gla protein also elaborated by mineralizing hypertrophic chondrocytes) are concomitantly upregulated. Importantly, TNAP activity is enhanced in NPP1−/− cultures.17 This mineralizing chondrogenic response is due to reduced extracellular PPI, because deficiency in ANK, the anionic transporter that helps control extracellular PPI concentration through export of cytoplasmic PPI,15 also potentiates endochondral vascular calcification.17 The paracrine regulation of mesenchymal cell fate by PPI is relevant to other tissue venues,11,12,14-16 as emphasized in their studies of bone marrow stromal cells (BMSCs).17 BMSCs are multipotent mesenchymal progenitors that exhibit molecular features of pericytic VSMCs.2,3,18 When cultured ex vivo with identical fibroblastic CFU yields, calcified nodule formation is markedly enhanced in cultures of NPP1−/−/H11001/H11002 BMSCs as compared with NPP1+/+ controls.17 Importantly, treatment of NPP1−/− BMSCs with
2.5 nM PPi not only suppressed mineral deposition but also concomitantly suppressed expression of the endochondral ossification program. Thus these data add considerable support to the authors’ accumulating evidence that PPi in fact is an active signaling molecule important in the paracrine regulation of apatitic calcium deposition in any context, whether it be in bone, osteoarthritic cartilage and ligament, or vascular calcification (Figure). Extracellular PPi, phosphate, and calcium ions thus interact with the better appreciated peptidyl paracrine and matricrine cues to guide lineage allocation of mesenchymal progenitors. Demer et al previously identified expression of the powerful bone morphogen BMP2 in calcifying vascular cells (CVCs); however, BMP2 protein levels are not discernibly different in wild-type versus NPP1/H11002/H11002 vascular myofibroblasts. How then might PPi signal to inhibit cartilaginous metaplasia and chondrogenic vascular calcium accumulation? Insight might be gained from the work of Price and colleagues. In a rigorous and systematic analysis, they identified that bisphosphonates, nonhydrolyzable pyrophosphate analogues, function as secretagogues for matrix Gla protein (MGP):fetuin complexes. MGP is a powerful modulator of transforming growth factor (TGF)-β superfamily signaling that is highly expressed in the vasculature. Importantly, Bostrom and colleagues have provided evidence that MGP inhibits BMP2 induction of TNAP, and MGP knockout mice develop massive arterial cartilaginous metaplasia and endochondral vascular calcification during postnatal development. Another mineralization inhibitor produced by VSMCs is osteopontin (OPN); in its phosphorylated form, OPN inhibits VSMC-mediated calcium deposition in vitro. Although deficiency in OPN does not result in cartilaginous metaplasia, it delays the egress of ectopic tissue calcification in vivo, and aged male OPN/H11002/H11002/ApoE/H11002/H11002 mice exhibit exaggerated vascular calcification. Intriguingly, Terkeltaub et al demonstrate that OPN protein secretion is markedly reduced in NPP1/H11002 cultures. Thus, given these observations, is it tempting to speculate that PPi regulates cartilage metaplasia through the secretion of proteinaceous extracellular matrix proteins.
Regulation of vascular calcification by inorganic pyrophosphate. Extracellular vascular PPI arises primarily from the activity of the ectoenzyme NPP1, with contributions from the cellular PPI exporter ANK. PPI inhibits calcification in part by biophysical properties that inhibit mineral apposition. However, Terkeltaub and colleagues also demonstrate that PPI inhibits cartilaginous metaplasia (chondrocytic "transdifferentiation") of VSMCs; with progression of the chondrocyte phenotype, alkaline phosphatase (TNAP, akp2) is upregulated. PPI is degraded by hydrolysis catalyzed by TNAP, an enzyme normally expressed at very highly levels only in osteoblasts, odontoblasts, and hypertrophic chondrocytes of the mineralizing skeleton. PPI also maintains expression of OPN, an inhibitor of vascular calcium accumulation. Whether PPI controls the production of other inhibitors (such as the MGP-fetuin complex) is as yet unknown, and the effect of PPI on VSMC osteogenic differentiation has yet to be studied.

Of note, loss of vascular NPP1, as occurs with NPP1 deficiency, or induction of vascular TNAP, as occurs in response to oxidized lipids, has the potential to result in a "feed-forward" cycle that could propagate the vascular calcification program. Nevertheless, these data clearly demonstrate that PPI is a regulator of vascular calcification and smooth muscle physiology. As such, PPI emerges as a novel vascular paracrine factor, of great potential significance to the calcific vasculopathy of diabetes, dyslipidemia, and end-stage renal disease.

Acknowledgments

D.A.T. is supported by National Institutes of Health grants HL69229, AR43731, and the Barnes-Jewish Hospital Foundation.

References

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doi: 10.1161/01.ATV.0000158943.79580.9d
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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