Osteogenic regulation of vascular calcification: an early perspective

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Vattikuti, Radhika, and Dwight A. Towler. Osteogenic regulation of vascular calcification: an early perspective. Am J Physiol Endocrinol Metab 286: E686–E696, 2004; 10.1152/ajpendo.00552.2003.—Cardiovascular calcification is a common consequence of aging, diabetes, hypercholesterolemia, mechanically abnormal valve function, and chronic renal insufficiency. Although vascular calcification may appear to be a uniform response to vascular insult, it is a heterogenous disorder, with overlapping yet distinct mechanisms of initiation and progression. A minimum of four histoanatomic variants—atherosclerotic (fibrotic) calcification, cardiac valve calcification, medial artery calcification, and vascular calciphylaxis—arise in response to metabolic, mechanical, infectious, and inflammatory injuries. Common to the first three variants is a variable degree of vascular infiltration by T cells and macrophages. Once thought benign, the deleterious clinical consequences of calcific vasculopathy are now becoming clear; stroke, amputation, ischemic heart disease, and increased mortality are portended by the anatomy and extent of calcific vasculopathy. Along with dystrophic calcium deposition in dying cells and lipoprotein deposits, active endochondral and intramembranous (nonendochondral) ossification processes contribute to vascular calcium load. Thus vascular calcification is subject to regulation by osteotropic hormones and skeletal morphogens in addition to key inhibitors of passive tissue mineralization. In response to oxidized lipids, inflammation, and mechanical injury, the microvascular smooth muscle cell becomes activated. Orthotopically, proliferating stromal myofibroblasts provide osteoprogenitors for skeletal growth and fracture repair; however, in valves and arteries, vascular myofibroblasts contribute to cardiovascular ossification. Current data suggest that paracrine signals are provided by bone morphogenetic protein-2, Wnts, parathyroid hormone-related polypeptide, osteopontin, osteoprotegerin, and matrix Gla protein, all entrained to endogenous calcification. During vascular injury and remodeling into endochondral, nonendochondral, or mixed ossification mechanisms, vascular calcification is associated with a characteristic spectrum of vascular disease processes. Moreover, in the setting of the calcified atherosclerotic plaque and senile calcific aortic sclerosis, the initial dystrophic calcification process evolves during vascular injury and remodeling into endochondral, nonendochondral, or mixed ossification mechanisms.

HISTOPATHOLOGY OF VASCULAR CALCIFICATION

Vascular calcium deposition can be usefully organized into four histoanatomic variants (Table 1). As outlined, each type of vascular calcification is associated with a characteristic spectrum of vascular disease processes. Moreover, in the setting of the calcified atherosclerotic plaque and senile calcific aortic sclerosis, the initial dystrophic calcification process evolves during vascular injury and remodeling into endochondral, nonendochondral, or mixed ossification mechanisms.

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Atherosclerotic (Fibrotic) Calcification

Atherosclerotic (fibrotic) calcification is a type of dystrophic calcification initially characterized by cellular necrosis, inflammation, and lipoprotein and phospholipid complexes (4, 22). The “endoarteritis deformans” of Virchow provides an excellent example of calcification in injured vascular tissue undergoing degenerative fibrotic calcification. Lipid complexes, derived from cellular membranes, adluminal thrombo-fibrinoid complexes, and serum lipoproteins nucleate calcium deposition in association with atherosclerotic plaques and old myocardial infarcts. Endothelial cell dysfunction provides a thrombogenic surface providing fibrin and platelet phospholipids and fibrin deposition that drive additional lipid deposition. Oxidized lipid products provide several signals that recruit and activate macrophages and T cells. Calcification is first noted in the lipid core of fibrocalcific plaques, juxtaposed to inflammatory cell infiltrate and cellular necrosis. Calcified cartilage formation follows the degenerative tissue calcification via a vascular remodeling process that deposits endochondral bone.

Cardiac Valve Calcification

Cardiac valve calcification occurs in response to mechanical stressors and inflammation, which recruit dystrophic mineralization and nonenchondral ossification processes to deposit calcium (endochondral ossification contributes to a lesser extent). Degenerative lipid accumulation, fatty expansion of the valvular fibrosa (mesenchymal tissue resembling adventitia), and stippled interstitial calcium deposition are accompanied by a macrophage and T cell infiltrate that is observed at the earliest stages of disease (72, 113). Of interest, interstitial cell inflammation and reactivity are also observed in calcifying bicuspid aortic valves in individuals without evidence of atherosclerosis (113). Thus, as recently considered (32, 70, 100, 116), valve calcification of the aged is likely initiated via mechanisms overlapping yet distinct from atherosclerotic calcification. During disease progression, however, histological and molecular analyses clearly demonstrate that a secondary phase of active, osteogenic mineral deposition contributes to continued vascular calcium accumulation, principally via nonenchondral processes (61, 80).

Medial Artery Calcification

Medial artery calcification is the nonenchondral ossification process of the arterial tunica media, highly characteristic of diabetes (14) and end-stage renal disease (see Vascular Calciphylaxis). The mineralization resembles intramembranous calvarial bone formation and odontogenesis (42), in that 1) no cartilaginous precursor is required; 2) bone morphogenetic protein-2 (BMP2)-Msx2-dependent signaling is a central feature of the mineralization process (16, 92, 110); and 3) electron micrographic studies demonstrate that mineralization initially occurs at matrix vesicles associated with extracellular matrix fibrils (104). Although not yet studied in the setting of renal insufficiency, diabetes upregulates aortic expression of Msx2 and Msx1 (16, 110). These two related homeodomain proteins are BMP2-regulated osteoblast transcription factors necessary for intramembranous ossification and odontogenic mineralization during craniofacial development (86). In pa-
tients with diabetes, both medial calcification and intimal atherosclerotic (degenerative) calcification can be individually visualized in the femoral artery (50). Importantly, medial calcification has emerged as the more significant predictor of lower extremity amputation and cardiovascular mortality risk in type 2 diabetes (50, 66).

The molecular determinants of medial calcification vs. atherosclerotic calcification with cartilage metaplasia are not known. Clues might be gleaned from comparisons of the vascular calcification responses of low-density lipoprotein receptor-null (LDLR−/−) mice (20, 92, 110) vs. apolipoprotein E-null (apoE−/−) mice (7, 57). Both models develop atheroma; however, LDLR−/− mice calcify valves and tunica media via a nonendochondral osteogenic process, whereas apoE−/− mice calcify vessels via cartilage metaplasia. Perhaps most importantly, unlike the LDLR−/− mouse, the apoE−/− mouse does not develop diabetes in response to high-fat diets (90). Data from our lab have revealed that an adventitial myofibroblast cell population is activated by diet-induced diabetes and is diverted to the osteoblast lineage by Msx2-dependent transcriptional programming (16, 92, 110). We hypothesize that a migratory adventitial cell myofibroblast population (85), responding to vascular smooth muscle cell (VSMC) osteopontin (OPN) production (3, 51, 110), contributes to vascular remodeling and the medial calcification of diabetes (see subsequent sections and Fig. 1) and, potentially, end-stage renal disease (15). Of note, both hyperglycemia (3, 110) and hyperphosphatemia (15) induce OPN expression, and a consistent feature of medial calcification is the medial expression of OPN (60, 91, 103) (see subsequent sections). Although not yet systematically tested, Bujan et al. (9) showed that surgical resection of the adventitia prevents segmental medial artery calcification in a rat model.

In mice, the absence of the osteoprotegerin (OPG) gene also results in medial calcification (no cartilage metaplasia) with vascular T cell infiltration and a high-turnover osteoporosis syndrome (8). Circulating OPG levels are increased in diabetic humans (6) and diabetic LDLR−/− mice (92) and may indicate a state of resistance to OPG actions. Inhibition of the receptor for activation of NF-κB (RANK) ligand signaling is a known, key feature of OPG action, but the relationships between this bioactivity and medial calcification are unclear (89).

Fig. 1. Medial vs. atherosclerotic calcification: working models for diabetic vasculopathy. Both medial and atherosclerotic types of calcification are a common occurrence in diabetes, arising independently in the same or anatomically similar arterial segments, but most often without overlap (50, 66). A: diabetes rapidly induces adventitial inflammation and oxidative stress and macrophage and T cell infiltration, and it upregulates expression of TNF-α, bone morphogenetic protein (BMP)2, Msx2, and osteopontin (OPN) gene expression. BMP2 and Msx2 expression occurs in adventitial pericytic myofibroblasts. OPN expression is upregulated in medial VSMCs, where expression enhances adventitial myofibroblast (osteoprogenitor) migration, proliferation, medial thickening, and matrix metalloproteinase (MMP)-dependent matrix turnover. Medial involvement with calcification is approximately concentric. The phosphorylation state of vascular OPN is unknown but will determine activity as a potent inhibitor vs. weak stimulus of VSMC mineral deposition. B: fibrous atherosclerotic plaque is intimal in orientation and asymmetric. Calcification occurs first at the necrotic lipid core. The fibrocalcific plaque elicits endochondral ossification responses, transcriptionally programmed by expression of Runx2 and Sox9. BMP2, which can drive both osteogenic and chondrogenic differentiation from mesenchymal progenitors, is expressed in a myofibroblast cell population. Oxidative stress of microvascular endothelial cells also upregulates BMP2. Multipotent calcifying vascular cells (CVCs) of Demer represent a subpopulation (10%–30%) of proliferative VSMCs. A smaller subset of medial myofibroblasts can be seen to express Msx2. Whether these cells represent migrating adventitial myofibroblasts, cells differentiating from mural CVCs, or even circulating mesenchymal progenitors that “home” to injured vasculature tissue has yet to be determined. See text for further details and citations.
Vascular Calciphylaxis

Vascular calciphylaxis is a component of the widespread soft tissue calcification that occurs when the physiological calcium phosphate solubility threshold is exceeded (79). The concentrations of calcium and phosphate are physiologically perched at levels within reach of the solubility product. Clinically, when the serum-calcium-phosphate product exceeds 60 mg²/dl², widespread tissue deposition of amorphous calcium phosphate can occur. Because of this, it appears that a diverse array of mineralization inhibitors has evolved, including fetuin-A, tissue pyrophosphate generating systems, and OPN. Fetuin-A (α2-Heremens-Schmid glycoprotein), an abundant serum glycoprotein, has recently been shown to limit organ and soft tissue calcification, including vascular calcium deposition, in a murine deficiency model (87). Of note, tissue calciphylaxis mediated by vitamin D is associated with downregulation of circulating fetuin-A (77). Pyrophosphate inhibits nucleation and epitaxial calcification and also upregulates the expression of osteopontin (38) (see subsequent sections). The generation of tissue pyrophosphate occurs via a family of three ectonucleotide pyrophosphatase/phosphodiesterases (ENPPs). ENPP1 (also known as PC-1) plays an important role in limiting calcification in “soft tissues,” such as ligaments and tendons (38). As elegantly described by Rutsch et al. (84), infants lacking ENPP1 develop spontaneous aortic calcification in addition to periarticular calcifications. Because cardiac valves also experience tremendous shear stress, exacerbated by bicuspid anatomy and hypertension, it is intriguing to speculate that age-related decrements in valvular ENPP1/PC-1 activities may contribute to degenerative dystrophic calcification. The control of vascular ENPP1/PC-1 by aging, diabetes, uremia, vascular inflammation, or endocrine cues is virtually uncharacterized.

Cartilage Metaplasia in Vascular Calcification

During vertebrate development, bone formation occurs via both endochondral and nonendochondral mechanisms (42). In endochondral ossification, an initially avascular cartilaginous template undergoes vascular invasion, cartilage calcification, osteoclast-dependent remodeling, and finally bone deposition by osteoblasts. Endochondral bone formation and neovascularization are highly dependent on expression of the transcriptional regulator, Runx2 (also known as Cbfa1 or Osf2), acting in concert with Sox9 (42, 119). The molecular fingerprints of endochondral ossification are commonly associated with the progression of degenerative atherosclerotic calcification (111). In two models of heritable vascular calcification, the matrix Gla protein (MGP) knockout mouse (54) and the apoE knockout mouse (57), arterial cartilage metaplasia occurs. The mechanisms are not entirely clear. Zebboudj et al. (118) demonstrated that MGP functions as a nogggin-like inhibitor of vascular BMP2 signaling. BMP2:BMP receptor II (BMPRII) inductive signaling is crucial to cardiovascular development (88). In the absence of MGP, dysregulated BMP2 signaling may drive vascular cartilage metaplasia. Loss of the intracellular BMPRII signaling inhibitor Smad6 (inhibitory vascular Smad) also results in arterial cartilage metaplasia and medial enchondral bone formation with marrow, but only in the aortic outflow track (28). Of note, BMP2 signaling can drive both chondrogenic and osteogenic differentiation of multipotent mesenchymal progenitors (93); in this setting, sustained Msx2 expression promotes osteogenic differentiation while excluding adipocytic (16) and chondrocytic (102) fates.

DYSMETABOLIC STIMULI AND VASCULAR CALCIFICATION: HYPERCHOLESTEROLEMIA, HYPERGLYCEMIA, AND HYPERPHOSPHATEMIA

The link between hypercholesterolemia, lipid oxidation, and atherosclerosis is robust, well documented, and well recognized (65). Only recently, however, has a connection been made between elevated LDL cholesterol and perturbed calcium homeostasis (74). Noting the positive correlation between postmenopausal osteoporosis and atherosclerotic calcification, Parhami, Demer and colleagues (59, 75, 108) examined the role of oxidized LDL cholesterol on calcifying vascular cellular (CVC) activity in vitro (Fig. 1). They identified that oxidized LDL upregulates CVC osteogenic mineralization and differentiation, but only in concert with physical cell-cell interactions between CVCs and macrophages (108). Osteogenic differentiation of CVCs could be recapitulated by inducing oxidative stress (75). Intriguingly, oxidative stress concomitantly suppressed osteoblast differentiation, indicating reciprocal cell type-specific responses (75). Thus the activated, cholesterol-laden foam cell participates in generating multiple osteogenic signals, potentially mediated by TNF-α and oxidized lipids. Of note, TNF-α has been shown to be a stimulus for mesenchymal BMP2 expression (27, 52), and macrophages may themselves produce BMP2 (12).

The hyperglycemia of diabetes generates metabolic, osmotic, and oxidative stresses. Although both atherosclerotic and medial types of calcification are common in diabetes (36), medial artery calcification is a highly characteristic vascular injury response to diabetes (14, 50, 66, 69). Once thought benign, medial artery calcification alters vascular compliance and has emerged as a significant risk factor for amputation and cardiovascular mortality (50, 66, 69). Even in the setting of chronic renal insufficiency, poor glycemic control is a very strong, independent predictor of vascular calcification (36). Clues to molecular mechanisms are emerging. As outlined, diabetes upregulates aortic adventitial BMP2-Msx2 osteogenic signaling cascades (16, 92, 110). Both glucose-regulated (58) and TNF-stimulated (52) vascular myofibroblasts are likely contributors to vascular BMP2 levels.

An important biological feature is the role for the tunica adventitia in diabetic vascular disease (110, 121). Adventitial genomic responses to diabetes have been known for almost two decades; in the 1980s, Lehmann et al. (49) showed by use of acridine orange histochemistry that both diabetes and hypercholesterolemia increased cells with euchromatic (active) character in the adventitia. In 1998, we (110) demonstrated that diet-induced diabetes, independent of atheroma formation, upregulated an Msx2-demarcated osteogenic gene regulatory program in a subset of adventitial myofibroblasts. Expression of the multifunctional cytokine OPN was also markedly upregulated by diabetes in the adventitia and tunica media (110). This past year, Zhang et al. (121) showed that streptozotocin-induced diabetes caused oxidative stress and an adventitial inflammatory response, the latter characterized by increased expression of TNF-α, a stimulus for BMP2 expression. Both direct glucose actions and advanced glycosylation end products are responsible for changes in vascular gene expression (3).
Because vascular OPN mediates myofibroblast proliferation (37), migration (51), and matrix metalloproteinase-dependent arterial remodeling (7, 37), we propose that diabetes-induced adventitial inflammation and tunica media OPN expression synergize to recruit osteoprogenitors to the tunica media during medial artery calcification (Fig. 1). This working model is being directly tested.

ENDOCRINE AND PARACRINE REGULATION OF VASCULAR CALCIUM METABOLISM

As is evident from the above discussion, homeostatic control of metabolic excesses by endocrine or pharmacological means holds promise for an effective, multipartite approach to the prevention and treatment of vascular calcification. For sevalamer (an oral phosphate binder that also lowers cholesterol), good clinical evidence already exists for efficacy (10, 17, 18). Whereas evidence exists that tight glucose control precludes diabetic microvascular disease, evidence that improved glucose control ameliorates macrovascular disease or vascular calcification is lacking (82). However, emerging data suggest that calcitropic hormones and osteogenic growth factors control vascular calcium metabolism in addition to skeletal mineral homeostasis; an overview is provided in this next section. The reader is referred to several excellent reviews by Demer, Parhami and colleagues (59, 73, 75, 108) that provide insights into the mechanisms whereby lipid metabolism and oxidation control calcific vasculopathy.

BMP2-Msx2 Signaling in Vascular Calcification

In vivo expression studies have documented the elaboration of chondrogenic and osteogenic transcriptional regulatory programs in the arterial tree during atherosclerotic calcification and diabetic vascular calcification (16, 110, 111). However, Bostrom et al. (4) were the first to highlight the expression of BMP2, a powerful bone morphogen, in calcifying atherosclerotic plaques, thus providing robust molecular evidence that an active osteogenic process contributes to vascular calcification (4). BMP2 is expressed by pericytic myofibroblasts, with expression in cells surrounding a highly calcified center. BMP2 induces both Msx2 and Runx2/Cbfal, the former associated with intramembranous bone formation and the latter with endochondral bone formation and neovascularization (42). The signals that initiate regional osteogenic and chondrogenic differentiation arise in part from lipid and glucose metabolism and inflammatory cytokines (12, 27, 58, 93, 108). Data from our lab demonstrated that aortic adventitial and vavular interstitial myofibroblast cell populations are activated by diabetes; the aortic myofibroblasts respond to BMP2-Msx2 signaling and are diverted to the osteoblast lineage by Msx2-dependent transcriptional programming (16, 110). This occurs because Msx2 upregulates osterix, or Osx, a global transcriptional regulator of mineralization and osteoblast differentiation (64), via a Runx2-independent signaling mechanism (Ref. 16 and data not shown). By contrast, in atherosclerotic calcification, Msx2 expression is also observed, but in a distinct subset of arterial myofibroblasts; however, Runx2-dependent endochondral ossification appears to predominate (111).

BMP2-Msx2 signaling promotes the osteogenic differentiation of vascular myofibroblasts (16). Of note, the microvascular myofibroblast, the pericyte, has emerged as a multipotent mesenchymal stem cell of adult tissues recruited to the osteogenic lineage during active phases of vascular calcification. Bright et al. (5) first demonstrated that retinal pericytes could undergo osteogenic differentiation in culture. Tintut et al. (107) extended these observations to the identification and characterization of CVCs, a subset of aortic VSMCs phenotypically similar to pericytes. Approximately 10–30% of mural VSMCs are CVCs (107). Doherty et al. (26) discovered that pericytes express Stro-1, a characteristic marker of the bone marrow stromal cell osteoprogenitor. Indeed, on the basis of anatomical, local Stro-1 expression, mesenchymal lineage potential, and VSMC α-actin expression, the bone marrow stromal cell can be considered a tissue-specific pericyte (109). One major difference appears to be emerging between CVCs and pericytes (Fig. 2); unlike pericytes (26) and adventitial myofibroblasts (16), CVCs apparently cannot differentiate into the adipocyte under standard conditions (107). The relative contributions of migratory adventitial pericytic myofibroblasts programmed by Msx2 (51, 85, 110, 121), transdifferentiation of resident medial CVCs (107), or even circulating skeletal progenitors (47) to osteogenic vascular calcification have yet to be clarified. Of note, Bujan et al. (9) demonstrated that surgical resection of the adventitia can prevent segmental medial artery calcification.

Endochondral bone formation and neovascularization are highly dependent on expression of the master transcriptional regulator Runx2, also known as Cbfal or Osf2 (42, 119). Because BMP2 signaling 1) up-regulates Runx2 as well as Msx2, and 2) can drive both chondrogenic and osteogenic differentiation of multipotent mesenchymal progenitors (93), BMP2-regulated processes will likely contribute all histoanatomical variants of vascular calcification. However, depending upon the disease process and the primary mechanisms driving vascular ossification (Table 1), the relative contributions of BMP2-Msx2 vs. BMP2-Runx2 signaling will likely differ.

![Fig. 2. Lineage potential and transcriptional programming of vascular osteoprogenitors. Pericytes, adventitial myofibroblasts, VSMCs, and CVCs all express VSMC α-actin. Multipotent CVCs of Demer represent a subpopulation (10–30%) of proliferative mural VSMCs. Recent data suggest that, unlike pericytes (26) and adventitial myofibroblasts (16), CVCs cannot differentiate into the adipogenic lineage under standard conditions (107). Msx2 promotes osteogenic differentiation while suppressing adipogenesis and chondrogenesis. Runx2 promotes endochondral bone formation. CCAAT enhancer-binding protein (CEBPα) and CEBPβ promote adipogenic differentiation, including expression of peroxisome proliferator-activated receptor (PPARγ). PPARγ suppresses osteogenic differentiation while promoting adipogenesis. See text for details.](http://ajpendo.physiology.org/ Downloaded from by 10.220.33.6 on June 23, 2017)
Like BMP2, BMP4 is expressed in diseased aortas (25), binds to the same BMP receptor and antagonist cohort as BMP2 (43), upregulates Msx2 gene expression in mesenchymal cells, and induces bone formation (46, 62). Sorescu et al. (96) recently showed that oscillatory shear stress upregulates BMP4 production by endothelial cells (96). Such BMP signaling mechanisms may contribute to paracrine regulation of vascular tone and remodeling via BMPRII, particularly notable in the pulmonary vasculature (21). However, endothelial stimulation with BMP4 or shear stress also induces an intercellular adhesion molecule (ICAM)-1-dependent inflammatory response, a feature common to macrovascular calcification arising from dysmetabolic state, advanced age, or mechanical stressors (61, 71, 72, 113). Thus, as a BMPR agonist, endothelial BMP4 expression may supply yet another paracrine signal that promotes both inflammation and osteogenic differentiation of arterial mesenchymal progenitors in response to mechanical insult. This intriguing observation (96) provides further insight into mechanisms whereby hypertension and bicuspid aortic valve promote macrovascular calcification (35, 71, 100, 113).

**Estrogen and Inflammatory Cytokines**

Within the last decade, an explosion of data has led to the realization that the actions of estrogen on calcium homeostasis occur in greatest part via the estrogen receptor-α (ESR1)-dependent control of paracrine signals that regulate T-cell expansion and expression of TNF-α (22, 45, 101, 105). In addition, stromal cell production of OPG, the negative regulator of the osteoclastogenic cytokine RANK ligand, is enhanced by estrogen tone (44). Thus, in response to the diminished estrogen tone of the menopause, a high-turnover skeletal metabolic state arises, with ensuing bone loss. Intriguingly, epidemiological studies have revealed that a reciprocal relationship exists between skeletal and vascular calcium accumulation; the dysregulated T cell cytokine milieu with estrogen withdrawal may represent a common pathophysiological feature (22, 45, 101, 105). The T cell cytokine RANK ligand has been shown to promote aortic valve myofibroblast calcification, an activity inhibited by OPG (40a). In mammographic studies, absence of prior hormone replacement therapy was strongly associated with increased radiographic arterial calcification (19). However, as recently learned from the Women’s Health Initiative, a progestin-estrogen hormone replacement combination does not prevent cardiovascular mortality (56) and stroke (114) and, in fact, increases risk. Thus the role for ESR1 signaling in calcific vasculopathy has yet to be unambiguously clarified in relevant clinical settings.

**Vitamin D and PTHrP**

The association of vitamin D toxicity with calcific vasculopathy has long been appreciated. However, recent data suggest a biphasic “dose-response” curve, with adequate vitamin D nutrition also being important for cardiovascular health (33, 94). By enhancing gastrointestinal absorption of calcium, the most active form of vitamin D, 1,25(OH)2D3, also known as calcitriol, elevates serum-ionized calcium. The direct and indirect suppression of parathyroid gland PTH output diminishes urinary phosphate excretion, with a net elevation in serum calcium, phosphate, and calcium-phosphate product. Thus vascular calciphylaxis represents one mechanism whereby vitamin D toxicity contributes to calcific vasculopathy. Indeed, vitamin D toxicity in rats is one common animal model of vascular calcification elicited in concert with warfarin (76) or nicotine (67). In a very enlightening study, Jono et al. (39) provided novel insight into calcitriol-mediated calcific vasculopathy. Vascular smooth muscle cells undergo calcification when treated in vitro with calcitriol, associated with the upregulation of alkaline phosphatase and suppression of PTHrP (39). Remarkably, simultaneous treatment of calcifying cultures with PTHrP dose-dependently suppressed alkaline phosphatase expression and calcium deposition. Confirming the role for active PTH1R signaling in this process, Jono et al. demonstrated that the antagonist PTHrP-(7–34) in fact enhanced alkaline phosphatase expression and calcium deposition. Therefore, excessive calcitriol exposure may suppress paracrine mechanisms that serve to defend the vasculature from calcification. In addition, activated macrophages express a constitutive 1α-hydroxylase and synthesize calcitriol from 25-hydroxyvitamin D; this could serve to limit the extent of tissue inflammation (1, 31, 78) and promote calcified granuloma formation to wall off infectious agents, as suggested by Demer and colleagues (59, 73, 75, 108). In certain disorders (sarcoidosis, tuberculosis, fungal infections), the extent of calcitriol production from granuloma can cause clinically significant hypercalcemia (29). Thus it is intriguing to speculate that calcification associated with vascular inflammation might progress in part via paracrine action of immunocyte calcitriol that locally suppresses the myofibroblast PTHrP defense.

**PTH and Teriparatide Signaling**

The data of Jono et al. (39) provided direct evidence that pharmacological manipulation of the PTH/PTHrP receptor (PTH1R) could regulate vascular calcification. Recently, we (92, 110) developed and evaluated an animal model of diet-induced diabetic vascular calcification. LDLR−/− mice fed high-fat diets characteristic of modern western societies develop insulin-resistant diabetes, hypercholesterolemia, and aortic calcification, first notable on cardiac valve leaflets. In situ hybridization and RT-PCR studies demonstrated the elaboration of a BMP2-Msx2 osteogenic gene regulatory program (92, 110). Msx2 expression was first noted in a subset of aortic adventitial cells and valve interstitial fibroblasts; on the basis of localization of VSMC α-actin, these cells appeared to be myofibroblasts. Ex vivo, these cells clearly respond to BMP2 with the upregulation of Msx2 and alkaline phosphatase (16). Consistent with these observations, transduction of myofibroblasts with Msx2 markedly enhances osteogenic potential (16). Armed with the above, we examined the influences of PTH-(1–34), teriparatide, on the diabetic vascular calcification associated with BMP2-Msx2 osteogenic differentiation, applying both in vivo and in vitro models. We identified once again a reciprocal relationship between orthotopic bone formation and vascular calcium accumulation. PTH-(1–34) enhanced skeletal mineral mass and upregulated skeletal osteopontin gene expression. By contrast, cardiovascular calcium deposition, OPN accumulation, and aortic Msx2 expression were concomitantly downregulated in this model (92). Thus, consistent with the in vitro studies of Jono et al., pharmacological stimulation of the PTH1R by PTH-(1–34) could prevent osteogenic vascular calcification.

Minireview

VASCULAR CALCIUM METABOLISM

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calcification at the initiation of diabetic vasculopathy. Ex vivo, we demonstrated that both PTH-(1–34) and OPN could inhibit vascular calcium accumulation and osteogenic gene expression in vitro. Because PTH-(1–34) upregulates circulating OPN levels (92), and OPN limits vascular calcium deposition in vivo and in vitro (40, 97, 99) (see subsequent sections), the combination of these two signaling pathways potentially contributes to an endocrine axis that serves to limit vascular calcium deposition in response to PTH-accentuated bone metabolism. The relative contributions of OPN and PTH-(1–34) are now being tested in OPN-null mice. The recent identification of the PTH2R (55) introduces the notion that some vascular responses to PTH derivatives may be mediated through this second receptor type. Of note, to date no study has adequately addressed contributions of PTH signaling to initiation vs. progression phases of vascular calcification; once the vascular osteogenic tissue has progressed to form mature bone tissue, PTH administration has the theoretical potential to augment mineral deposition and turnover via its osteoanabolic actions. Moreover, a direct comparison of the effects of PTH on atherosclerotic (which has an endochondral ossification) and calcifying soluble OPN levels, it in fact suppresses OPN gene expression and matrix-associated protein accumulation in cardiovascular tissues (92). This suggests that the soluble form of OPN exerts direct actions on vascular mesenchymal cells that actively suppress mineral deposition, in addition to the matrix-crine activities that promote monocyte/macrophage activation. However, only intact OPN was assayed (92), and no quantitative assessment of OPN phosphorylation in vascular or circulating forms induced by PTH has yet been performed. Because phosphorylation is a key regulatory step relevant to either inhibiting (phospho-OPN) or promoting (dephospho-OPN) VSMC calcification (40), strategies are being developed to address this shortcoming.

END-STAGE RENAL DISEASE: A PERFECT STORM IN VASCULAR CALCIFICATION

Phosphate retention and accumulation of PTH fragments that perturb normal calcium phosphate homeostasis drive tremendous vascular calcium loads in the setting of end-stage renal disease (ESRD). As is evident in the setting of calciphylaxis, the thermodynamic mass action effects of increased serum phosphate levels can drive widespread calcium phosphate deposition. Thus the phosphate retention of ESRD presents opportunity for intervention but confounds simple interpretation of disease pathophysiology and progression. Because of its multiplicate didactic nature, calcific vasculopathy of ESRD is deserving of its own mention (81).

Twenty million Americans have measurably impaired renal function, and three million individuals have clinically relevant kidney disease (30). As a consequence of diabetes, hypertension, glomerulonephritis, and hereditary developmental abnormalities, ESRD has a prevalence of ~95,000 new cases per annum; in the US, ~300,000 individuals with ESRD undergo hemodialysis, peritoneal dialysis, or transplantation. The annual mortality is ~75,000 per year from ESRD. As highlighted by Goodman et al. (30), vascular calcium deposition is severe in this patient population. The reasons for this are manifold. Diabetes, hypercholesterolemia, hypertension, and hyperphosphatemia all tip the balance toward vascular calcium deposition. As stated above, Jono et al. demonstrated that paracrine vascular PTHrP limits VSMC calcification (39); thus the widespread use of calcitriol to limit secondary and tertiary hyperparathyroidism may exert unintended deleterious consequences on vascular calcium load via suppression of vascular PTHrP. Indeed, recent data using a selective vitamin D receptor agonist, paricalcitol, demonstrate improved mortality compared with traditional calcitriol therapy (106). Although beneficial actions on calcium-phosphate product have been monitored, actions of paricalcitol on vascular PTHrP expression are unknown. Recently, Langub et al. (48) identified a proteolytic fragment of PTH, PTH-(7–34), that accumulates in ESRD and functions to induce resistance to PTH. Elegant work by Sned-
don et al. (95) has now shown that PTH-(7–84) binds the PTH1R and does not elicit signaling cascades, but it downregulates cell surface expression by enhancing dynamin-dependent internalization. Thus, if PTH1R signaling plays important roles in promoting skeletal mineral accumulation while simultaneously limiting vascular calcium accumulation, the accumulation of such antagonistic PTH fragments may contribute to the calcific vasculopathy of ESRD. Expression of sodium hydrogen exchanger regulatory factor (NHERF)1 prevents PTH (7–84)-dependent PTH1R internalization (95); thus downregulation of NHERF1 expression in ESRD would contribute to the blunted PTH sensitivity of uremia via enhanced cell-surface PTH1R endocytosis. These novel features of PTH1R biology as relevant to vascular and skeletal metabolism have yet to be studied in the clinically relevant setting of ESRD.

SUMMARY, UNRESOLVED ISSUES, UNMET NEEDS, AND FUTURE DIRECTIONS

The interplay between metabolic, inflammatory, and mechanical stressors, passive mineralization inhibitors, and calcitrophic hormones determines the fate of multipotent vascular progenitors that regulate vascular calcium load. Although vascular calcification may appear to be a uniform response to vascular insult, it is a heterogenous disorder, with overlapping yet distinct mechanisms of initiation, histopathological progression, and clinical consequences. A large number of issues have yet to be resolved concerning the regulation of vascular calcification. A detailed understanding of the origins of the chondro- and osteoprogenitors needs to be established. The relative contributions of transdifferentiating VSMCs and migratory adventitial myofibroblasts to vascular calcification responses needs to be determined. In addition, it is probable that a “homing response” induced by vascular injury will recruit circulating marrow skeletal progenitors (47) and contribute to disease progression, including the development of ectopic vascular bone marrow in association with vascular osteogenesis (11, 120). Better cell surface markers of the mesenchymal lineage are required. The role of the omnipresent T cell in vascular calcification is poorly understood and may help to explain the reciprocal relationships between bone turnover and vascular calcification. Given the limitations of animal models, it will be important to extend key observations of vascular calcium metabolism to patient-oriented translational research. To do so, better noninvasive imaging strategies will need to be implemented to adequately resolve, categorize, and quantify each histoanatomic form of vascular calcification (Table 1). With cardiac cycle gating, sensitive volumetric vascular calcium measurements by multislice spiral computed tomography may help to address this need (34, 68). Data from several processes; the net effect on vascular health is unknown. The roles and regulation of MMPs in dystrophic tissue remodeling and vascular calcification are unexplored. Noninvasive strategies for dynamic imaging of vascular calcium influx, calcium egress, MMP activation, and vascular matrix turnover are required. Finally, in the setting of ESRD, hyperglycemia, hypertension, hypercholesterolemia, hyperphosphatemia, PTH resistance, and iatrogenic calcitriol excess may all contribute to vascular calcium load (30, 36). Abnormal phosphate homeostasis in the setting of ESRD markedly alters the severity, progression, and extent of vascular calcification and may do so even if hyperphosphatemic exposure is relatively transient and episodic. Because BMP2 is observed at high levels adjacent to dystrophic calcification, dystrophic tissue calcium phosphate deposition may serve as a metabolic stressor that induces BMP2 expression. Molecules engineered to inhibit BMP signaling (115) may serve clinically useful roles for limiting the heterotopic vascular calcification associated with vascular protheses (98). As our understanding of vascular calcium metabolism and endocrine regulation matures, novel strategies for prevention and treatment of this clinically important, multifaceted vasculopathy will no doubt emerge.

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