Phosphate and Vascular Calcification: Emerging Role of the Sodium-Dependent Phosphate Cotransporter PiT-1

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Summary

Elevated serum phosphate is a risk factor for vascular calcification and cardiovascular events in kidney disease as well as in the general population. Elevated phosphate levels drive vascular calcification, in part, by regulating vascular smooth muscle cell (VSMC) gene expression, function, and fate. The type III sodium-dependent phosphate cotransporter, PiT-1, is necessary for phosphate-induced VSMC osteochondrogenic phenotype change and calcification, and has recently been shown to have unexpected functions in cell proliferation and embryonic development.

Keywords

phosphate; vascular calcification; PiT-1; kidney disease

Introduction

Vascular calcification, the inappropriate deposition of calcium phosphate salts in valvular and vascular tissue, occurs at high prevalence in aging, diabetes, and renal disease. In blood vessels, intimal calcification is associated with atherosclerotic plaques and coronary artery occlusion, whereas arterial medial calcification (AMC) is a non-occlusive process that leads to stiffening of vessels (1).

There is a heavy burden of cardiovascular disease in the chronic kidney disease (CKD) population. The prevalence is twice that of the general population, and advances at twice the rate (2). In the US, the one-year mortality following an acute myocardial infarction is 46% in CKD patients *vs* 26% in those without CKD (3). Using electron beam CT imaging, Braun et al. documented a 2- to 5-fold increase in coronary artery calcification in dialysis patients, compared with age-matched non-CKD patients with known coronary artery disease (4). Both intimal and medial calcification occur in the elastic arteries of CKD patients to a greater extent than in age-matched controls (5).

AMC in particular is an important cause of decreased vascular compliance in aging, diabetes, and CKD (6). In AMC, hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ is deposited in the arterial media (7). Pediatric patients present a unique opportunity to examine vascular calcification in CKD in the absence of atherosclerotic risk factors (smoking, hypertension, diabetes, dyslipidemia). Vessels from pediatric dialysis patients show entirely medial calcification, without evidence of inflammation or atherosclerosis (8). Increased vascular stiffness translates into increased pulse wave velocity and pulse pressures, both of which are highly associated with increased mortality in the dialysis population (9). Clinically,

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increased vascular stiffness leads to hypertension, left ventricular hypertrophy, heart failure and compromised coronary perfusion (10). At the arteriole level, AMC causes the ischemic skin lesions characteristic of calcific uremic arteriolopathy, a condition observed in dialysis patients that carries an extremely high mortality rate (11).

Phosphate balance, vascular calcification and cardiovascular disease

Inorganic phosphorus, in the form of phosphate, is a critical mineral found in bone, phospholipids and nucleic acids; and is required for high-energy phosphate bonds, intracellular signaling and pH buffering. In humans, 85% of total body phosphorus is in bone, 14% is intracellular and only 1% is in extracellular fluid (12). The kidney is the major regulator of phosphate homeostasis, whereby 70% of phosphate reabsorption in the proximal convoluted tubule occurs via the sodium-dependent phosphate cotransporter (NaPi)- IIa (6). Two other phosphate transporters, NaPi-IIc and phosphate transporter (PiT)-2, also contribute to renal phosphate reabsorption (13).

The phosphatonins, or hormonal regulators of phosphate balance, include Vitamin D3, fibroblast growth factor 23 (FGF-23) with its cofactor klotho, and parathyroid hormone (PTH) (6). Active vitamin D3 ($1,25[OH]_2$ D3 or calcitriol) is a steroid hormone primarily synthesized in the kidneys, and is decreased in CKD (14). NaPi-IIb of the duodenal enterocyte is a transcriptional target of the vitamin D receptor (15), but calcitriol deficiency in CKD does not result in decreased intestinal phosphate uptake due to passive phosphate transport through enterocyte paracellular pathways (6). FGF-23/klotho decrease phosphate reabsorption by the NaPi-IIa transporter, and inhibit vitamin D3 production via suppression of 1α-hydroxylase (16). Uremic mice with early CKD demonstrate elevated circulating FGF-23 and are able to maintain normal serum phosphate levels (17). It is suggested that klotho acts in a paracrine fashion, where the extracellular domain is shed from the distal convoluted tubule and secreted in the adjacent proximal convoluted tubule where FGF-23 is expressed. Secondary hyperparathryoidism in CKD is a consequence of decline in calcitriol production, hypocalcemia and hyperphosphatemia. There is parathyroid hyperplasia and increased PTH synthesis, with resultant dysregulated bone remodeling (6). FGF-23 and PTH increase urinary phosphate excretion, however these defense mechanisms are ineffective as renal function continues to fall in advanced CKD. Hence, hyperphosphatemia in CKD is a consequence of decreased phosphate excretion as well as disordered bone remodeling (renal osteodystrophies) (6).

Elevated serum phosphate has emerged as a non-traditional risk factor for cardiovascular events in CKD (18,19), as well as in the general community (20). In a study of veterans with CKD, a statistically significant increase in mortality risk was noted with serum phosphate levels >3.5 mg/dl (21). The high risk of mortality is likely mediated, in part, by increased vascular calcification in CKD patients. This was demonstrated in prospective, randomized clinical trials where treatment with a dietary phosphate-binder attenuated vascular calcification (22,23) and mortality in dialysis patients (23). However, other randomized trials have not confirmed mortality and quality-of-life benefits with lowering of serum phosphate in dialysis patients (24,25), emphasizing the complexity of mineral metabolism of which there is still much to learn.

The role of phosphate in vascular calcification has come under intense investigation in the past decade. The vascular smooth muscle cell (VSMC) is now known to play a prominent role in phosphate-induced calcification, and recent data has implicated the involvement of elastin degradation.

Mechanisms of phosphate-induced vascular calcification

Regulation of VSMC fate: Osteochondrogenic phenotype change versus apoptosis

When the clinical significance of hyperphosphatemia surfaced in the late 1990s, it spurred bench research that reinforced phosphate's role in AMC. *In vitro*, no matrix calcification occurs in human VSMCs incubated with 1.4 mM phosphate (physiological level), but dose-dependent calcification occurs when phosphate levels are increased from 1.6 mM to 3 mM (26). Subsequently, Giachelli's group studied phosphate loading using a uremic mouse whereby a two-step surgery involving partial electrocautery of the right kidney and total nephrectomy of the left kidney results in moderate to severe uremia (17). Uremic mice fed a high (0.9%) phosphate diet developed robust AMC without atherosclerosis or inflammation. Treatment with a dietary phosphate-binder (sevelamer) attenuated phosphate-driven vascular calcification in this model (unpublished findings), consistent with findings in uremic rats (27) and in CKD patients (28).

There are two major hypotheses regarding fate of VSMCs in phosphate-induced vascular calcification. The first invokes a profound transition to a bone-forming phenotype. *In vitro* studies have shown that elevated phosphate results in loss of smooth muscle markers (SM alpha actin, SM22alpha) and expression of osteochondrogenic markers (Runx2/Cbfa1, osterix, alkaline phosphatase, osteopontin) (26,29). Runx2/Cbfa1 is a transcription factor that induces the expression of major components of the bone matrix including type I collagen, osteocalcin, and osteopontin. In addition, ultrastructural analysis of the surface of VSMCs shows matrix vesicles containing apatite which act as nucleation sites for calcification (similar to the vesicles that bud from osteoblasts and hypertrophic chondrocytes), as well as calcifying collagen fibrils (30). Evidence for *in vivo* VSMC lineage reprogramming to the bone-forming phenotype has been found in calcified vascular lesions from animals (17,31,32) and humans (8). The complexity of VSMC phenotype change was recently underscored using whole-genome expression arrays of uremic rats fed on a high phosphate diet; the transition from "muscle-related" to "bone-related" gene expression involved dysregulation of 53 genes (33). Activation of extracellular signal-regulated kinases (Erks) has been implicated; mouse aortic medial cells treated with high phosphate demonstrate increase of phosphorylated Erk1/2 levels along with increase of Runx2/Cbfa1, prior to loss of VSMC lineage markers (32). The canonical Wnt pathway, known to be important in bone formation, is also likely to be involved in phosphate-induced vascular calcification. Secreted frizzled related proteins (SFRPs) inhibit the Wnt pathway, and SFRPs-1, 2 and 4 are upregulated in uremic rats fed on a high phosphate diet presumably as a defensive response against further ossification (33).

The second hypothesis involves apoptosis-dependent matrix mineralization. Reynolds et al. cultured human VSMCs in elevated phosphate and calcium conditions, and this led to calcification of matrix vesicles and apoptotic bodies (34). Arteries from pediatric dialysis patients show VSMC loss due to apoptosis with release of matrix vesicles from damaged/dead VSMCs (8). These findings were replicated in a recent study where vessel rings from pediatric subjects were cultured under various calcium/phosphate concentrations (35). Vessels from healthy controls were resistant to calcification whereas dialysis vessels developed dense medial calcification in association with apoptosis, cell loss and deposition of apatite-containing vesicles. Growth arrest-specific gene 6 (Gas6) is believed to exert anti-apoptotic effects via the Bcl2-mediated PI3K-AKT pathway (36). Gas6 is markedly downregulated along with its receptor Axl in phosphate-induced VSMC calcification (37); phosphorylation inactivates Bcl2 and activates Bad, resulting in caspase-3 activation and apoptosis (36). Also, bone morphogenetic proteins have been shown to downregulate Bcl2 in pulmonary VSMCs (38).

These mechanisms are not mutually exclusive, as recently proposed by Shanahan's group (8,35). It is likely that VSMCs undergo osteochondrogenic adaptive changes, moving from a "contractile" to a "secretory" phenotype and deposit matrix vesicles to protect the cell from the toxic effects of elevated calcium and phosphate. However, when this system becomes overwhelmed and as matrix calcification propagates, cell death ensues.

Regulation of inducers and inhibitors of hydroxyapatite formation

Phosphate may additionally promote vascular calcification by modulating levels of inducers and inhibitors of hydroxyapatite formation that are synthesized by VSMCs. For example, tissue non-specific alkaline phosphatase (TNAP) is an ectoenzyme present on cell surfaces and in matrix vesicles that is absolutely required for bone formation (7,39). Inorganic pyrophosphate consists of two molecules of phosphate linked by an ester bond, and is a key inhibitor of hydroxyapatite formation (7). TNAP cleaves pyrophosphate thus generating more phosphate, and is upregulated *in vitro* in phosphate-driven VSMC calcification (26,29). Rat aortic rings incubated with elevated phosphate calcify only when subjected to mechanical damage, and express elevated TNAP and decreased pyrophosphate (40). Upregulation of TNAP occurs in dialysis patients (8,41) and could be partly due to dialysis-induced VSMC injury (8). TNAP also dephosphorylates osteopontin, another inhibitor of medial calcification that is upregulated but inactive in uremic conditions (17,26).

Elastocalcinosis

Besides VSMCs, the other major component of the tunica media is the elastic lamellae. Elastin degradation increases its affinity for calcium (42), facilitating epitactic growth of hydroxyapatite along the elastic lamellae. Elastin degradation has been implicated in phosphate-induced AMC by several studies. Uremic mice fed a high-phosphate showed calcified deposits initiating at sites of elastin degradation (17). Furthermore, Moreau et al. studied elastocalcinosis in cultured rat aortic rings in the presence of elevated phosphate and warfarin treatment (43). They found early expression of matrix metalloproteinase MMP-9, an elastin degrading enzyme, closely followed by TGF-β signaling and osteochondrogenic phenotype transition of VSMCs. Similarly, treatment of rat VSMCs with alpha-elastin accelerated phosphate-induced VSMC transformation (44). Simionescu et al. have shown that peptides derived from elastin degradation bind to elastin laminin receptors (ELRs) on the surface of adjacent VSMCs, and perpetuate proliferation and osteogenic differentiation via TGF-β signaling (45). TGF-β upregulates Runx2/Cbfa1, the major osteoblastic transcription factor (46,47). Of note, MMP knockout mice are resistant to elastin degradation and calcification (48). Finally, expression of matrix metalloproteinases MMP-2 and 9, two key elastase enzymes, are upregulated in arteries from diabetic CKD patients and correlate with vascular stiffness (49).

Role of PiT-1 in phosphate-induced vascular calcification

Phosphate transport into cells is primarily mediated by NaPi cotransporters, of which there are 3 types. The type I and II NaPi cotransporters in kidney and intestinal epithelium play important roles in whole body phosphate homeostasis; they are also present in other tissues including liver and brain (50,51). The type III NaPi cotransporters, PiT-1 and PiT-2, are ubiquitously expressed. Real-time PCR detects similar RNA levels of PiT-1 and PiT-2 in rat VSMCs, and there is no expression of type I nor type II NaPi cotransporters (52). In contrast, PiT-1 is the predominant NaPi cotransporter in human VSMCs (53).

PiT-1 (also known as gibbon ape leukemia virus receptor 1 [Glvr-1], leukemia virus receptor 1 homolog, solute carrier family 20 member 1 [SLC20A1]) has been identified as a pivotal transporter in phosphate-induced VSMC calcification. Jono et al. reported that phosphate

uptake in human VSMCs was dependent on a sodium gradient, and treatment with phosphonoformic acid (PFA, a competitive inhibitor of NaPi transport) inhibited phosphate uptake and VSMC osteochondrogenic differentiation (26). Real-time PCR and Northern blot analysis suggested that the NaPi cotransporter involved was PiT-1 (26). Further investigation was done using PiT-1 specific small interfering RNAs (siRNAs) (53). PiT-1 knockdown in human VSMCs suppressed phosphate-induced mineralization, and subsequent overexpression of mouse PiT-1 rescued phosphate transport and VSMC osteochondrogenic changes. Neither phosphate loading of matrix vesicles nor apoptosis was mediated by PiT-1. Taken together, these findings demonstrate that PiT-1 is required for phosphate-induced calcification of human VSMCs *in vitro*. Of note, it was recently shown that PFA prevents calcification by direct inhibition of hydroxyapatite formation (inhibits nucleation in a similar way to pyrophosphate and bisphosphonates (54)) and is a poor inhibitor of type III NaPi co-transporters (55). Future mechanistic studies involving this class of transporters should therefore be cautious with the use of PFA.

Interestingly, PiT-1 is upregulated by inducers of calcification including BMP-2 (56,57), calcium (58), and platelet-derived growth factor PDGF (59,60). Suzuki et al. exposed MC3T3-E1 osteoblast-like cells to BMP-2 and found an increase in the V(max) of Na-dependent phosphate transport (57). The aforementioned study by Jono et al. showed increasing phosphate uptake by human VSMCs as extracellular phosphate was raised from 0.5 mM to 3 mM (26). In contrast, Villa-Bellosta et al. found that PiT-1 and PiT-2 transporters in rat VSMCs are saturated under physiological phosphate concentrations (52). Indeed, this group has used membrane protein biotinylation to demonstrate that PDGF treatment of rat VSMCs increased PiT-1 abundance at the endoplasmic reticulum and not at the cell surface (59), discussed further in the next section. These disparate findings may be due to species variation. Overall, PiT-1 in vascular calcification likely involves more complex pathways beyond phosphate transport, as described in more detail below.

PiT-1 signaling involves Erk activation

As noted previously, treatment of calcifying mouse VSMCs with high phosphate results in increased phosphorylation of Erk 1/2 in conjunction with VSMC osteochondrogenic transdifferentiation (32). Inhibition of Erk phosphorylation by the MEK inhibitor U0126 prevented upregulation of Runx2/Cbfa1 and promoted VSMC lineage markers (32). Other studies have implicated Erks in VSMC regulation and osteoblast differentiation (61–63). Erk 1/2 signaling is also activated by the calcification inducer BMP-2 (61) and by platelet-derived growth factor-BB-mediated modulation of VSMC proliferation (62,64). PiT-1 may also exert effects at the endoplasmic reticulum level. Rat aortic VSMCs treated with PDGF show increased PiT-1 expression in the endoplasmic reticulum, as evidenced by co-localization with markers such as ERp46 and Derlin-1 (59). From these findings, Villa-Bellosta et al. hypothesize that PiT-1 may promote vascular calcification via modulation of anti-calcification proteins (such as MGP) or modification of kinases that phosphorylate secreted matrix proteins (such as osteopontin) (59).

Figure 1 outlines the putative roles of PiT-1 and the VSMC changes that occur in phosphate-mediated vascular calcification.

Other roles of PiT-1

The type III NaPi cotransporters were originally described as retroviral receptors with multiple membrane-spanning regions in the early 1990s (65,66). It later emerged that they were important for phosphate transport in mammalian cells (67,68). Besides mediating vascular calcification, PiT-1 and PiT-2 may be involved in important physiological pathways in bone, parathyroid glands, kidney, and intestine (69). Given that AMC mimics

bone formation, it is not surprising that PiT-1 has been implicated in bone development and mineralization. PiT-1 can be detected in 17-day old murine embryos in hypertrophic chondrocytes that are initiating mineralization (70). In osteoblast-like cells, PiT-1 expression increases during differentiation and correlates with mineralization (57).

PiT-1 and cell proliferation

Many processes and structures in the cell require phosphate. These include the phospholipids in the cell membranes, DNA, ATP production, and phosphorylation of proteins. Recently, Beck et al. demonstrated that knockdown of PiT-1 in HeLa and HepG2 cells resulted in decreased cell proliferation (71). Interestingly, the regulation of cell proliferation via PiT-1 did not depend on phosphate transport, as a PiT-1 protein defective in Pi transport rescued the proliferation defect in these cells (71). Thus, PiT-1 may have important roles in addition to cellular phosphate homeostasis.

Knockout of NaPi transporters

Genetic knockouts of various phosphate transporters have been used to understand the physiological role of these transporters. The double knockout of mouse NaPi-IIa and NaPi-IIc is viable, but synergistically results in severe hypophosphatemia and rickets, while the single gene knockouts do not (72). Murine NaPi-IIb knockout results in lethality between embryonic day (E) 8.0 and 10.5 and display defects in growth, somitogenesis, angiogensis, and failure of the chorionic trophoblasts to form the labyrinth layer in the placenta (73). Considering the role of PiT-1 in VSMC calcification in vitro, interest has developed in also studying the role of PiT-1 in an *in vivo* system. To that end, PiT-1 knockout mice have been recently created by two different groups. Surprisingly, like the NaPi-IIb mice, PiT-1 knockout was embryonic lethal, though at a different stage. PiT-1 null embryos arrested between E11.5 and 13.5 and displayed severe anemia (74,75). Anemia might be due to increased apoptosis and reduced proliferation of the fetal liver (75), but a fundamental defect in hematopoesis has not yet been ruled out. In addition, one group observed abnormal vascular development of the yolk sac (74), though this was not observed by the second group (75). Though the role of PiT-1 in bone development or calcification cannot be addressed in these knockout null embryos, the use of PiT-1 conditional alleles (74,75) will be invaluable to create tissue-specific knockouts and the use of the hypomorphic allele (75) will allow for the analysis of subtle phenotypes where the expression level of PiT-1 is critical. No PiT-2 knockout mice have yet been reported.

Conclusions

Serum phosphate has emerged as a non-traditional risk factor for vascular disease and mortality in CKD. The VSMCs from dialysis vessels are particularly susceptible to calcification due to prolonged exposure to the CKD milieu. Awareness of the importance of calcium-phosphate balance has led to renewed interest in comparing phosphate clearance of various dialysis modalities (76–78), and in patient education on dietary phosphorus content (79,80). More data is needed regarding oral phosphate binders (see (81) for review). The biology of the arterial tunica media is greatly altered in elevated phosphate conditions; there is VSMC transition to bone phenotype and apoptosis, inactivation of local anti-calcification factors, and elastin degradation. The PiT-1 phosphate transporter appears to be a key mediator in phosphate-induced VSMC osteochondrogenic differentiation, and was recently discovered to be important in cell proliferation and embryonic development, indicating more functions for this protein than previously thought.

Acknowledgments

References

1. London G, Marchais S, Guérin A, et al. Arteriosclerosis, vascular calcifications and cardiovascular disease in uremia. Curr Opin Nephrol Hypertens 2005 Nov;14(6):525–31. [PubMed: 16205470]

2. Collins A, Li S, Gilbertson D, et al. Chronic kidney disease and cardiovascular disease in the Medicare population. Kidney Int Suppl 2003 Nov;(87):S24–31. [PubMed: 14531770]

3. USRDS. United States Renal Data System Annual Data Report. 2009 [accessed on Oct 14, 2009]. http://www.usrds.org/adr.htm

4. Braun J, Oldendorf M, Moshage W, et al. Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. Am J Kidney Dis 1996 Mar;27(3):394–401. [PubMed: 8604709]

5. Ibels L, Alfrey A, Huffer W, et al. Arterial calcification and pathology in uremic patients undergoing dialysis. Am J Med 1979 May;66(5):790–6. [PubMed: 443254]

6. Hruska K, Mathew S, Lund R, et al. Hyperphosphatemia of chronic kidney disease. Kidney Int 2008 Jul;74(2):148–57. [PubMed: 18449174]

7. Demer L, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. Circulation 2008 Jun;117(22):2938–48. [PubMed: 18519861]

8. Shroff R, McNair R, Figg N, et al. Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis. Circulation 2008 Oct;118(17):1748–57. [PubMed: 18838561]

9. Blacher J, Safar M, Guerin A, et al. Aortic pulse wave velocity index and mortality in end-stage renal disease. Kidney Int 2003 May;63(5):1852–60. [PubMed: 12675863]

10. Vanholder R, Massy Z, Argiles A, et al. Chronic kidney disease as cause of cardiovascular morbidity and mortality. Nephrol Dial Transplant 2005 Jun;20(6):1048–56. [PubMed: 15814534]

11. Ahmed S, O'Neill K, Hood A, et al. Calciphylaxis is associated with hyperphosphatemia and increased osteopontin expression by vascular smooth muscle cells. Am J Kidney Dis 2001 Jun; 37(6):1267–76. [PubMed: 11382698]

12. Goldman, L.; Ausiello, D. Cecil Medicine. 23. Saunders Elsevier; 2007.

13. Villa-Bellosta R, Ravera S, Sorribas V, et al. The Na+-Pi cotransporter PiT-2 (SLC20A2) is expressed in the apical membrane of rat renal proximal tubules and regulated by dietary Pi. Am J Physiol Renal Physiol 2009 Apr;296(4):F691–9. [PubMed: 19073637]

14. Reichel H, Deibert B, Schmidt-Gayk H, et al. Calcium metabolism in early chronic renal failure: implications for the pathogenesis of hyperparathyroidism. Nephrol Dial Transplant 1991;6(3):162–9. [PubMed: 1866044]

15. Hattenhauer O, Traebert M, Murer H, et al. Regulation of small intestinal Na-P(i) type IIb cotransporter by dietary phosphate intake. Am J Physiol 1999 Oct;277(4 Pt 1):G756–62. [PubMed: 10516141]

16. Shimada T, Hasegawa H, Yamazaki Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res 2004 Mar;19(3):429–35. [PubMed: 15040831]

17. El-Abbadi M, Pai A, Leaf E, et al. Phosphate feeding induces arterial medial calcification in uremic mice: role of serum phosphorus, fibroblast growth factor-23, and osteopontin. Kidney Int 2009 Jun;75(12):1297–307. [PubMed: 19322138]

18. Block G, Hulbert-Shearon T, Levin N, et al. Association of serum phosphorus and calcium × phosphate product with mortality risk in chronic hemodialysis patients: a national study. Am J Kidney Dis 1998 Apr;31(4):607–17. [PubMed: 9531176]

19. Young E, Albert J, Satayathum S, et al. Predictors and consequences of altered mineral metabolism: the Dialysis Outcomes and Practice Patterns Study. Kidney Int 2005 Mar;67(3):1179–87. [PubMed: 15698460]

20. Dhingra R, Sullivan L, Fox C, et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med 2007 May;167(9):879–85. [PubMed: 17502528]

21. Kestenbaum B, Sampson J, Rudser K, et al. Serum phosphate levels and mortality risk among people with chronic kidney disease. J Am Soc Nephrol 2005 Feb;16(2):520–8. [PubMed: 15615819]

22. Chertow G, Burke S, Raggi P. Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. Kidney Int 2002 Jul;62(1):245–52. [PubMed: 12081584]

23. Block G, Raggi P, Bellasi A, et al. Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. Kidney Int 2007 Mar;71(5):438–41. [PubMed: 17200680]

24. Suki W, Zabaneh R, Cangiano J, et al. Effects of sevelamer and calcium-based phosphate binders on mortality in hemodialysis patients. Kidney Int 2007 Nov;72(9):1130–7. [PubMed: 17728707]

25. St Peter W, Liu J, Weinhandl E, et al. A comparison of sevelamer and calcium-based phosphate binders on mortality, hospitalization, and morbidity in hemodialysis: a secondary analysis of the Dialysis Clinical Outcomes Revisited (DCOR) randomized trial using claims data. Am J Kidney Dis 2008 Mar;51(3):445–54. [PubMed: 18295060]

26. Jono S, McKee M, Murry C, et al. Phosphate regulation of vascular smooth muscle cell calcification. Circ Res 2000 Sep;87(7):E10–7. [PubMed: 11009570]

27. Cozzolino M, Staniforth M, Liapis H, et al. Sevelamer hydrochloride attenuates kidney and cardiovascular calcifications in long-term experimental uremia. Kidney Int 2003 Nov;64(5):1653–61. [PubMed: 14531797]

28. Block G, Spiegel D, Ehrlich J, et al. Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. Kidney Int 2005 Oct;68(4):1815–24. [PubMed: 16164659]

29. Steitz S, Speer M, Curinga G, et al. Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. Circ Res 2001 Dec;89(12):1147–54. [PubMed: 11739279]

30. Wada T, McKee M, Steitz S, et al. Calcification of vascular smooth muscle cell cultures: inhibition by osteopontin. Circ Res 1999 Feb;84(2):166–78. [PubMed: 9933248]

31. Graciolli F, Neves K, dos Reis L, et al. Phosphorus overload and PTH induce aortic expression of Runx2 in experimental uraemia. Nephrol Dial Transplant 2009 May;24(5):1416–21. [PubMed: 19075196]

32. Speer M, Yang H, Brabb T, et al. Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. Circ Res 2009 Mar;104(6):733–41. [PubMed: 19197075]

33. Román-García P, Carrillo-López N, Fernández-Martín J, et al. High phosphorus diet induces vascular calcification, a related decrease in bone mass and changes in the aortic gene expression. Bone. 2009 Sep;

34. Reynolds J, Joannides A, Skepper J, et al. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. J Am Soc Nephrol 2004 Nov;15(11):2857–67. [PubMed: 15504939]

35. Shroff R, McNair R, Skepper J, et al. Chronic mineral dysregulation promotes vascular smooth muscle cell adaptation and extracellular matrix calcification. J Am Soc Nephrol 2010 Jan;21(1):103–12. [PubMed: 19959717]

36. Son B, Kozaki K, Iijima K, et al. Gas6/Axl-PI3K/Akt pathway plays a central role in the effect of statins on inorganic phosphate-induced calcification of vascular smooth muscle cells. Eur J Pharmacol 2007 Feb;556(1–3):1–8. [PubMed: 17196959]

37. Son B, Kozaki K, Iijima K, et al. Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring Gas6-Axl survival pathway. Circ Res 2006 Apr;98(8):1024–31. [PubMed: 16556867]

38. Zhang S, Fantozzi I, Tigno D, et al. Bone morphogenetic proteins induce apoptosis in human pulmonary vascular smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2003 Sep;285(3):L740–54. [PubMed: 12740218]

39. Murshed M, Harmey D, Millán J, et al. Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone. Genes Dev 2005 May; 19(9):1093–104. [PubMed: 15833911]

40. Lomashvili K, Cobbs S, Hennigar R, et al. Phosphate-induced vascular calcification: role of pyrophosphate and osteopontin. J Am Soc Nephrol 2004 Jun;15(6):1392–401. [PubMed: 15153550]

41. Lomashvili K, Garg P, Narisawa S, et al. Upregulation of alkaline phosphatase and pyrophosphate hydrolysis: potential mechanism for uremic vascular calcification. Kidney Int 2008 May;73(9): 1024–30. [PubMed: 18288101]

42. Rucker R. Calcium binding to elastin. Adv Exp Med Biol 1974;48(0):185–209. [PubMed: 4372871]

43. Bouvet C, Moreau S, Blanchette J, et al. Sequential activation of matrix metalloproteinase 9 and transforming growth factor beta in arterial elastocalcinosis. Arterioscler Thromb Vasc Biol 2008 May;28(5):856–62. [PubMed: 18292396]

44. Hosaka N, Mizobuchi M, Ogata H, et al. Elastin Degradation Accelerates Phosphate-Induced Mineralization of Vascular Smooth Muscle Cells. Calcif Tissue Int. 2009 Oct;

45. Simionescu A, Philips K, Vyavahare N. Elastin-derived peptides and TGF-beta1 induce osteogenic responses in smooth muscle cells. Biochem Biophys Res Commun 2005 Aug;334(2):524–32. [PubMed: 16005428]

46. Heldin C, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 1997 Dec;390(6659):465–71. [PubMed: 9393997]

47. Lee K, Kim H, Li Q, et al. Runx2 is a common target of transforming growth factor beta1 and bone morphogenetic protein 2, and cooperation between Runx2 and Smad5 induces osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2C12. Mol Cell Biol 2000 Dec;20(23):8783–92. [PubMed: 11073979]

48. Basalyga D, Simionescu D, Xiong W, et al. Elastin degradation and calcification in an abdominal aorta injury model: role of matrix metalloproteinases. Circulation 2004 Nov;110(22):3480–7. [PubMed: 15545515]

49. Chung A, Yang H, Sigrist M, et al. Matrix metalloproteinase-2 and -9 exacerbate arterial stiffening and angiogenesis in diabetes and chronic kidney disease. Cardiovasc Res. 2009 Aug;

50. Takeda E, Taketani Y, Morita K, et al. Sodium-dependent phosphate co-transporters. Int J Biochem Cell Biol 31(3–4):377–81. [PubMed: 10224663]

51. Werner A, Dehmelt L, Nalbant P. Na+-dependent phosphate cotransporters: the NaPi protein families. J Exp Biol 1998 Dec;201(Pt 23):3135–42. [PubMed: 9808829]

52. Villa-Bellosta R, Bogaert Y, Levi M, et al. Characterization of phosphate transport in rat vascular smooth muscle cells: implications for vascular calcification. Arterioscler Thromb Vasc Biol 2007 May;27(5):1030–6. [PubMed: 17322102]

53. Li X, Yang H, Giachelli C. Role of the sodium-dependent phosphate cotransporter, Pit-1, in vascular smooth muscle cell calcification. Circ Res 2006 Apr;98(7):905–12. [PubMed: 16527991]

54. Villa-Bellosta R, Sorribas V. Phosphonoformic acid prevents vascular smooth muscle cell calcification by inhibiting calcium-phosphate deposition. Arterioscler Thromb Vasc Biol 2009 May;29(5):761–6. [PubMed: 19213941]

55. Ravera S, Virkki L, Murer H, et al. Deciphering PiT transport kinetics and substrate specificity using electrophysiology and flux measurements. Am J Physiol Cell Physiol 2007 Aug; 293(2):C606–20. [PubMed: 17494632]

56. Li X, Yang H, Giachelli C. BMP-2 promotes phosphate uptake, phenotypic modulation, and calcification of human vascular smooth muscle cells. Atherosclerosis 2008 Aug;199(2):271–7. [PubMed: 18179800]

57. Suzuki A, Ghayor C, Guicheux J, et al. Enhanced expression of the inorganic phosphate transporter Pit-1 is involved in BMP-2-induced matrix mineralization in osteoblast-like cells. J Bone Miner Res 2006 May;21(5):674–83. [PubMed: 16734382]

58. Yang H, Curinga G, Giachelli C. Elevated extracellular calcium levels induce smooth muscle cell matrix mineralization in vitro. Kidney Int 2004 Dec;66(6):2293–9. [PubMed: 15569318]

59. Villa-Bellosta R, Levi M, Sorribas V. Vascular smooth muscle cell calcification and SLC20 inorganic phosphate transporters: effects of PDGF, TNF-alpha, and Pi. Pflugers Arch 2009 Oct; 458(6):1151–61. [PubMed: 19506901]

60. Kakita A, Suzuki A, Nishiwaki K, et al. Stimulation of Na-dependent phosphate transport by platelet-derived growth factor in rat aortic smooth muscle cells. Atherosclerosis 2004 May;174(1): 17–24. [PubMed: 15135246]

61. Gallea S, Lallemand F, Atfi A, et al. Activation of mitogen-activated protein kinase cascades is involved in regulation of bone morphogenetic protein-2-induced osteoblast differentiation in pluripotent C2C12 cells. Bone 2001 May;28(5):491–8. [PubMed: 11344048]

62. Wang Z, Wang D, Hockemeyer D, et al. Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. Nature 2004 Mar;428(6979):185–9. [PubMed: 15014501]

63. Xiao G, Jiang D, Thomas P, et al. MAPK pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbfa1. J Biol Chem 2000 Feb;275(6):4453–9. [PubMed: 10660618]

64. Heldin C, Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 1999 Oct;79(4):1283–316. [PubMed: 10508235]

65. Johann S, Gibbons J, O'Hara B. GLVR1, a receptor for gibbon ape leukemia virus, is homologous to a phosphate permease of Neurospora crassa and is expressed at high levels in the brain and thymus. J Virol 1992 Mar;66(3):1635–40. [PubMed: 1531369]

66. Miller D, Edwards R, Miller A. Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for gibbon ape leukemia virus. Proc Natl Acad Sci U S A 1994 Jan;91(1):78–82. [PubMed: 8278411]

67. Kavanaugh M, Miller D, Zhang W, et al. Cell-surface receptors for gibbon ape leukemia virus and amphotropic murine retrovirus are inducible sodium-dependent phosphate symporters. Proc Natl Acad Sci U S A 1994 Jul;91(15):7071–5. [PubMed: 8041748]

68. Olah Z, Lehel C, Anderson W, et al. The cellular receptor for gibbon ape leukemia virus is a novel high affinity sodium-dependent phosphate transporter. J Biol Chem 1994 Oct;269(41):25426–31. [PubMed: 7929240]

69. Collins J, Bai L, Ghishan F. The SLC20 family of proteins: dual functions as sodium-phosphate cotransporters and viral receptors. Pflugers Arch 2004 Feb;447(5):647–52. [PubMed: 12759754]

70. Palmer G, Zhao J, Bonjour J, et al. In vivo expression of transcripts encoding the Glvr-1 phosphate transporter/retrovirus receptor during bone development. Bone 1999 Jan;24(1):1–7. [PubMed: 9916777]

71. Beck L, Leroy C, Salaun C, et al. Identification of a novel function of PiT1 critical for cell proliferation and independent from its phosphate transport activity. J Biol Chem. 2009 Sep;

72. Segawa H, Onitsuka A, Furutani J, et al. Npt2a and Npt2c in mice play distinct and synergistic roles in inorganic phosphate metabolism and skeletal development. Am J Physiol Renal Physiol 2009 Sep;297(3):F671–8. [PubMed: 19570882]

73. Shibasaki Y, Etoh N, Hayasaka M, et al. Targeted deletion of the tybe IIb Na+-dependent Pi-co-transporter, NaPi-IIb, results in early embryonic lethality. Biochemical and Biophysical Research Communications 2009;381(4):482–6. [PubMed: 19233126]

74. Festing M, Speer M, Yang H, et al. Generation of mouse conditional and null alleles of the type III sodium-dependent phosphate cotransporter PiT-1. Genesis 2009 Dec;47(12):858–63. [PubMed: 19882669]

75. Beck L, Leroy C, Beck-Cormier S, et al. The phosphate transporter PiT1 (Slc20a1) revealed as a new essential gene for mouse liver development. PLoS One 2010;5(2):e9148. [PubMed: 20161774]

76. Walsh M, Manns B, Klarenbach S, et al. The effects of nocturnal compared with conventional hemodialysis on mineral metabolism: A randomized-controlled trial. Hemodial Int. 2009 Dec;

77. Davenport A, Gardner C, Delaney M. The effect of dialysis modality on phosphate control : haemodialysis compared to haemodiafiltration. The Pan Thames Renal Audit. Nephrol Dial Transplant 2010 Mar;25(3):897–901. [PubMed: 19875379]

78. Kuhlmann M. Phosphate elimination in modalities of hemodialysis and peritoneal dialysis. Blood Purif 2010;29(2):137–44. [PubMed: 20093819]

79. Ahlenstiel T, Pape L, Ehrich J, et al. Self-adjustment of phosphate binder dose to meal phosphorus content improves management of hyperphosphataemia in children with chronic kidney disease. Nephrol Dial Transplant. 2010 Mar;

80. Kalantar-Zadeh K, Gutekunst L, Mehrotra R, et al. Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. Clin J Am Soc Nephrol 2010 Mar;5(3):519–30. [PubMed: 20093346]

81. Navaneethan S, Palmer S, Craig J, et al. Benefits and harms of phosphate binders in CKD: a systematic review of randomized controlled trials. Am J Kidney Dis 2009 Oct;54(4):619–37. [PubMed: 19692157]
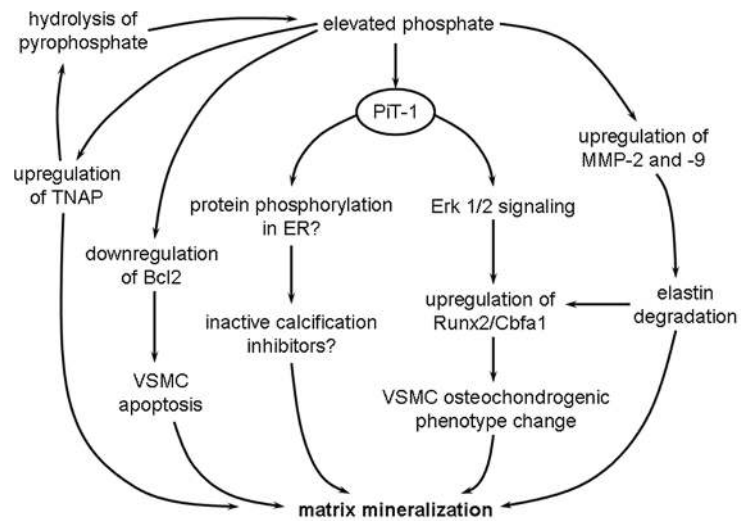
Figure 1.
Proposed mechanisms of phosphate-induced vascular calcification include vascular smooth muscle cell (VSMC) phenotype change, VSMC apoptosis, elastin degradation, and dysregulation of pro- and anti-calcification mediators. TNAP cleaves pyrophosphate to generate two molecules of phosphate, perpetuating elevated phosphate conditions. PiT-1 is necessary to induce VSMC osteochondrogenic differentiation, and may additionally play a role in protein modulation at the endoplasmic reticulum that results in inactivation of anti-calcification mediators such as osteopontin and matrix Gla protein, though evidence for this is needed. Whether PiT-1 is required for phosphate-driven apoptosis and/or elastin degradation is not yet known. ER endoplasmic reticulum; MMPs matrix metalloproteinases; PiT-1 type III sodium-dependent phosphate cotransporter 1; PPi pyrophosphate; TNAP tissue non-specific alkaline phosphatase.