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Where Do We Stand on Vascular Calcification?

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Abstract

Vascular disease, such as atherosclerosis and diabetic vasculopathy, is frequently complicated by vascular calcification. Previously believed to be an end-stage process of unregulated mineral precipitation, it is now well established to be a multi-faceted disease influenced by the characteristics of its vascular location, the origins of calcifying cells and numerous regulatory pathways. It reflects the fundamental plasticity of the vasculature that is gradually being revealed by progress in vascular and stem cell biology. This review provides a brief overview of where we stand in our understanding of vascular calcification, facing the challenge of translating this knowledge into viable preventive and therapeutic strategies.

Keywords

vascular calcification; review; signaling pathway; calcifying cells

1. Introduction

We are approaching an understanding of vascular calcification, which was described in exquisite detail already in the late 19th and early 20th century [1–3]. Those early studies predicted and posed the questions that are still actively being studied today. Ectopic bone formation was commonly found in the vascular wall, and included the presence of osteoblasts- and osteoclast-like cells, osteoid, and fully formed bone with marrow spaces. Furthermore, the proximity of calcified areas to penetrating capillaries was noted as well as “metaplasia” of connective tissue into bone tissue, suggesting links to stem cells and a relationship between vasculature and bone. These findings posed questions on the origin of the calcifying cells, whether derived from the vascular wall itself or the circulation, cells that had according to Bunting [1] retained “into late life embryonic characteristics” and were “capable of a diverse development under appropriate stimuli”.

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Vascular calcification frequently emerges as a complication of disorders such as atherosclerosis and diabetic vasculopathy, indicating that an imbalance in the vascular wall is a prerequisite for triggering calcification. It also suggests that vascular calcification could be restricted by controlling the factors that provoke vascular disease, such as hyperlipidemia, diabetes and hyperphosphatemia. Most likely, preventive treatments would be aimed at the entire vasculature and promote the overall cardiovascular health. However, there would also be situations where a targeted approach might be of value, such as a simple reversal of vascular calcification in a particular segment of a vessel or a cardiac valve. The epitome of this would be the softening of aortic valves by non-surgical methods, which has the potential of improving the quality of life for numerous individuals, reducing health care costs, and in some cases saving lives. However, at this time, there is no effective preventive or targeted treatment for cardiovascular calcification.

Several things need to be considered when developing interventions aimed at vascular calcification, including the type of vascular calcification, the origins of the calcifying cells, and the characteristics of signaling pathways that regulate vascular calcification. Here, we provide a brief summary of the vascular calcification field, to serve as a primer for additional studies.

2. Diversity of Vascular Calcification and Relationship to Vascular Disease

Initially, as vascular calcification became a topic of interest, all calcification was treated equally. However, it was soon recognized that some calcification existed in the form of remodeled ectopic bone, whereas other calcification consisted of mineralized matrix, still seemingly untouched by the remodeling forces of osteoblast- and osteoclast-like cells. Several patterns of calcification, which may exist in isolation or in combination, are currently known to exist and relate to various pathological conditions (Table 1). The calcification depends on the type of vessel, the disease that affects it, and what layer of the vascular wall is targeted by the disease. Basically all vascular layers can be affected by calcification, giving calcification a highly diverse face.

Systemic arteries

Most commonly, calcification affects thick-walled elastic arteries in the systemic circulation (Figure 1), which are the targets of atherosclerosis and media sclerosis. Atherosclerosis is an inflammatory disorder promoted by a number of different risk factors, including hyperlipidemia, hypertension, diabetes, and tobacco use [4]. The atherosclerotic lesions develop in the arterial intima, often at specific locations that are subjected to disturbed flow such as branch points [5]. The calcification usually occurs at the base of the lesion, in proximity to the media. The lesions may show evidence of fully remodeled bone, cartilage metaplasia, adipose tissue, and bone marrow elements [6]. For example, cartilage metaplasia is a well-known phenomenon in the innominate arteries of apolipoprotein E (*ApoE*) null mice [7].

Media sclerosis, also referred to as Mönckeberg's disease, is classically associated with diabetes, chronic kidney disease and aging [8]. The calcification occurs along the elastic lamellae in the media, but may also involve the internal elastic lamina [9]. Signs of

inflammation are rare in media sclerosis in contrast to atherosclerosis, although both frequently co-exist. How the absence versus the presence of inflammation influences calcification is poorly understood. Hyperphosphatemia, a hallmark of chronic kidney disease, is strongly associated with media sclerosis [8,10]. Pediatric patients with renal failure appear to be particularly sensitive to develop vascular calcification, possibly due to the still immature state of their vasculature. Systolic hypertension is usually worsened by the increased arterial stiffness associated with calcification, which in turn may further promote vascular osteogenesis. It has been shown that increased matrix rigidity can direct cells along the bone lineage [11], which would reinforce calcific and hypertensive changes.

Porcelain aorta is severe circumferential aortic calcification, which is limited to the ascending aorta and aortic arch and involves the aortic media [12]. It poses significant problems during cardiovascular interventions such as valve surgery by limiting cross-clamping of the ascending aorta. Interestingly, the location is similar to that of osteochondrogenesis observed in *Smad6* null mice [13]. Coral reef aorta is another rare type of calcification [14], where the calcifications protrude into the lumen, predominantly in the posterior thoracic and abdominal aorta.

Pulmonary arteries

The pulmonary arteries are less affected by vascular calcification than the systemic arteries, likely due to a variety of reasons, such as exposure to lower blood pressure in the pulmonary circulation. Indeed, calcification of the pulmonary artery is a known consequence of longstanding pulmonary artery hypertension [15].

Arterioles

Calcific uremic arteriolopathy, also referred to as calciphylaxis, is a rare but serious disease that occurs mainly in patients with end-stage renal disease. It obliterates the lumen of small arteries and arterioles, and may result in life-threatening soft tissue necrosis. Recent data suggest that it involves an osteogenic process driven by bone morphogenetic protein (BMP)2 signaling [16].

**Cardiac Valves **

One of the clinically most significant types of calcification is aortic valve calcification associated with the development of aortic stenosis. It has many similarities to vascular calcification in regards to regulation [17], but may also exhibit features unique to the valves. Aortic valve calcification is dramatic, often occurring in the form of bulky nodules on the aortic side of the aortic cusps with fusion of the valve cusps and decreased aortic valve opening. Although rarely seen nowadays, similar calcification may occur in the pulmonary valves, provided that the patient survives into adulthood with severe pulmonic stenosis without valve replacement [18].

Myocardium

Already in 1924, myocardial calcification was extensively reviewed [19], and is now a topic that is gathering increasing interest. In this type of calcification, the mineral is found in the myocardium itself and affects the contractile myocardium as well as the conduction system.

The mechanisms are poorly understood but includes a number of risk factors such as previous myocardial infarction and renal failure [20]. Currently, the clinical awareness of this process is less than that of vascular and valvular calcification, although it has severe consequences for the patient.

3. Sources of Calcifying Cells

Several sources of cells that undergo osteochondrogenic differentiation in the vascular wall have been identified (Table 2), and might differ depending on the type of vascular calcification. Initially, since calcification is predominantly seen in the media, all focus was on medial cells, including vascular smooth muscle cells (SMCs) and pericytes. The focus then widened and included cells from all the vascular layers, including the adventitia and the endothelium [21]. Ultimately, the stem cell field brought concepts of stemness, vascular niches, and endothelial-mesenchymal transitions (EndMTs) that merged with the vascular calcification field and gave us a wide range of options in regards to calcifying cell origin. It also gave us insight into the considerable plasticity that exists in vascular cells. Even though all sources may contribute calcifying cells, the fraction derived from each source is unclear, and may differ between diseases and vascular beds.

a. Medial cells

Synthetic, dedifferentiated, or “phenotype-switched” SMCs are major contributors to neointima formation, and appear to be responsive to osteogenic cues. Such cues are found in for example atherosclerotic lesions, and diabetic and hyperphosphatemic states. Once the media has been disturbed and the elastic lamellae degraded, the normally quiescent layers of SMCs and intimal cells undergo either a dedifferentiation to less mature cells, followed by osteochondrogenic redifferentiation, or a direct transdifferentiation from SMCs to osteochondrogenic cells [22]. Calcifying subpopulations have been isolated from aortic media [23], and may represent cells in transition or stable clones of SMCs.

b. Pericytes

Pericytes cover the capillary endothelial tubes and provide a similar support function as the SMCs. They are well known to take on osteogenic characteristics if provided the right stimuli [24].

c. Endothelial cells

Aortic and valvular endothelial cells (ECs) have been shown to transition to cells undergoing osteochondrogenesis through EndMTs [25,26]. The EndMT was associated with an increase in stem cell-like properties, suggesting that a certain level of dedifferentiation of the ECs occurred prior to the osteochondrogenic differentiation [26], rather than direct transdifferentiation from endothelial to osteogenic cells. The occurrence of EndMTs is a strong indication that “stemness” is situational and context-dependent, and places ECs in line for targeting by anti-calcific therapies.

d. Adventitial cells

On the external side of the vascular wall, myofibroblasts have been implicated in osteogenic programs initiated in the adventitia in hyperlipidemic and diabetic animals [27]. Adventitial myofibroblasts are highly mobile and can migrate into inflamed areas of the media. It has not been addressed whether they have a relationship to vasa vasorum or neoangiogenesis originating in the same layer.

e. Progenitor cells

The revelation of calcifying cells in the vasculature paralleled in part the development of the stem cell field. Vascular wall progenitor cells may be stationary in the vascular wall, transported through the circulation, or migrating directly into the vascular tissue [28]. Bone marrow-derived stem circulating stem cells have been shown to contribute to osteoblastic as well as osteoclastic cells in the artery wall [29]. Another potential source of stem cells are vascular niches, with preferred locations in the adventitia [30], where stemness is regulated by events in the niches. Thus, progenitor cells contribute plasticity to the vasculature that could start to explain its malleability in various tissues and organs.

f. Osteoclast-like cells

Osteoclast-like cells have been detected in the vascular wall, and would be an important, possibly targetable, aspect of vascular calcification due to their ability to degrade mineral and regulate osteoblastic cells. These cells may be derived from monocytic cells in the circulation that are triggered to undergo osteoclastic differentiation in the calcific milieu of the diseased vascular wall [31].

4. Signaling Pathways and Relation of Calcific Disease

Multiple regulatory axes have been implicated in the development of vascular calcification (Figure 2). One way to understand the various stimuli influencing vascular calcification is to divide them into predominantly activating or inhibitory stimuli. In some cases, there is a direct connection between a pro-calcific activator and its inhibitory partner through feedback regulation or physical interaction. Activating and inhibitory stimuli may work in parallel or in a temporal sequence, in some cases with a spatial component, such as the destruction of normal vascular wall layering. Naturally, the regulatory axes will in turn form an interactive network, complicated by the intertwining of intra- and extra-cellular factors, which together will influence the calcific response. Below is an abbreviated list of regulatory signaling systems known to influence vascular calcification. New regulatory factors are continuously added, sometimes modulating these major axes.

a. Bone morphogenetic protein (BMP) signaling

The BMPs constitute a sub-group of the TGF β superfamily of growth factors [32,33], which is essential in vascular development, remodeling, and disease. Several of the BMPs are potent activators of osteogenic differentiation, and were among the earliest factors described in the calcified artery wall [23], suggesting vascular calcification to be a regulated process. The BMPs frequently have cell-specific actions, and their effect on vascular calcification depends in part on where and by what cells they are expressed. BMP activation has been

associated with atherosclerosis, diabetic vasculopathy, chronic kidney disease and high levels of phosphate [34–36], conditions that promote calcification.

BMP2 appears to have a special role in promoting calcification in the vascular media, where it acts at least in part through Runx2 (Cbfa1) and microRNA-30b and 30c [36,37]. BMP2 further participates in a program in adventitial myofibroblasts that involves Msx2 and LRP6 in diabetic and hyperlipidemic mice [38,39] and may mediate pro-calcific effects of hyperphosphatemia and nanocrystals [36,40]. BMP4, although closely related to BMP2, enhances vascular calcification by actions in the endothelium in mouse models of vascular calcification caused by deficiency of matrix Gla protein (MGP) and diabetes [41]. BMP4 has been shown to mediate endothelial inflammation as well as EndMTs [26,42]. Interestingly, BMP7, which is functionally similar to BMP4 during development, protects against vascular calcification in chronic kidney disease [43].

Inhibition of BMP signaling can be achieved by naturally occurring inhibitors such as MGP, which functions as a calcification inhibitor. The importance of MGP was revealed by *Mgp* gene deletion in mice, which resulted in ossification of the elastic arteries [44]. In mice, MGP has been demonstrated to prevent EndMT in the aortic endothelium [41] and transdifferentiation of medial SMCs to osteochondrogenic cells [22]. Abnormal calcification has also been detected in human MGP deficiency, referred to as Keutel syndrome [45]. Conversely, enhanced MGP expression limits atherosclerotic and diabetic calcification in mice [34,35], further testifying to the importance of BMP inhibition.

MGP regulates BMP through direct protein-protein interactions, and binds calcium through gamma-carboxylated glutamate residues [46]. It appears to have a dual function in regulating BMP activity and protecting mineral nucleation on elastin sites by binding to mineral crystals [46,47]. Interestingly, a decrease in aortic elastin content diminishes the calcification in the *Mgp* null mouse [47]. It is not yet clear how the different MGP functions relate to the calcification process, but may involve transglutaminase 2 and elastin fragmentation [48]. Interference with the gamma-carboxylation of MGP, which is susceptible to warfarin, might be a link between clinical warfarin use and vascular calcification [49].

Additional BMP inhibitors have been shown to inhibit vascular calcification. Fc-fragments directed against the activin receptor-like kinase (ALK)3, a BMP receptor, reduces atherosclerotic calcification in fat-fed *LDL receptor* (*Ldlr*) null mice [50], and the small molecule BMP inhibitor LDN-193189 prevents vascular calcification in the *Mgp* null mice [51]. The deletion of Smad6, an inhibitory transcription factor in the BMP signaling cascade, also results in osteochondrogenesis [13].

b. Wnt signaling

The Wnt signaling system is highly complex [52], and both activators and inhibitors of Wnt signaling affects the development of vascular calcification. LRP6, a Wnt receptor, limits osteochondrogenic differentiation in vascular SMCs in *Ldlr* null mice, fed a high-fat diet to induce diabetic vasculopathy [39]. On the other hand, Dkk1, a Wnt antagonist, enhances EndMTs and calcification in aortic ECs, whereas Wnt7b in cooperation with the Msx2

transcription factor tries to maintain a stable EC phenotype [53], which reduces the conversion of cells to the osteochondrogenic lineage.

c. Phosphate signaling

Close regulation of phosphate levels is paramount for vascular health and involves several strong activators and inhibitors of calcification. Nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) synthesizes extracellular inorganic pyrophosphate (ePPI), a potent calcification inhibitor, from ATP released by cells during mineralization. Mutations in the *Npp1* gene lead to an overwhelming early vascular calcification, referred to as generalized arterial calcification of infancy (GACI) [54]. GACI causes heart failure and death early in life, sometimes even prenatally, and may be considered an extreme form of hyperphosphatemia where all counterbalance provided by ePPI is removed. Tissue-nonspecific alkaline phosphatase (TNAP) hydrolyzes and eliminates ePPI and overexpression of this enzyme also leads to vascular calcification due to enhanced phosphate levels [55]. ATP-binding cassette sub-family C member 6 (ABCC6) appears to be the primary source of the ATP used by NPP1 [56], a connection that could explain the ectopic calcification in ABCC6 deficient mice and humans.

Hyperphosphatemia is also a consequence of progressive renal failure, which leads to impaired renal secretion of phosphate and stimulation of vascular calcification [8,10]. The fibroblast growth factor 23 (FGF-23) promotes phosphate excretion through the kidneys, thereby limiting the pro-calcific actions of phosphate. Klotho, which exists as a transmembrane protein and in soluble form, functions as a co-factor to FGF-23. Deficiency of FGF-23 or Klotho, results in similar phenotypes that include diffuse vascular calcification [57,58]. Chronic kidney disease also correlates with abnormalities in the parathyroid hormone and Vitamin D signaling [8,10], which can further promote the pro-calcific state in renal failure.

d. Signaling in diabetes mellitus

High glucose affects calcification through multiple systems, of which BMP signaling has already been mentioned. In addition, the receptor for advanced glycosylation end products (RAGE) has been implicated in vascular calcification in diabetes [59]. It mediates signaling by S100A12 and other RAGE ligands, and connects with signaling involving oxidative stress and NADPH oxidase (Nox), other promoters of vascular calcification [59,60]. Recently, studies by Heath et al. revealed new potential targets in diabetic vascular calcification. Their studies showed the importance of AKT activation by O-linked N-acetylglucosamine for promoting vascular calcification [61]. In addition, they showed that phosphatase and tensin homolog (PTEN), a negative regulator of AKT, limits diabetic calcification [61,62].

e. Osteoprotegerin – RANK/RANKL

Gene deletion of osteoprotegerin (OPG) causes both severe vascular calcification and osteoporosis [63]. Together with receptor activator of nuclear factor-kappaB (RANK) and RANK ligand (RANKL), OPG forms a fundamental regulatory system of osteoclast formation that plays an important role in bone turnover and potentially in the remodeling and removal of mineral deposits in the vasculature [57]. OPG appears to have a limiting

effect on vascular calcification, whereas RANKL is believed to promote vascular calcification. However, there are still inconsistencies in the precise role of the vascular OPG-RANK/RANKL axis, such as the finding of increased serum levels of OPG in the presence of vascular calcification [64]. OPG has also been found to limit the action of TNF-related apoptosis-inducing ligand (TRAIL), a strong activator of apoptosis [65], which may create niches for calcification.

f. Matrix vesicles / exosomes

When VSMCs undergo osteochondrogenic conversion in response to pro-calcific stimuli, they release specialized membrane-bound bodies referred to as matrix vesicles (MV), which nucleate calcium phosphate crystals in the form of hydroxyapatite [66]. The MVs released from VSMCs were recently identified as exosomes originating from intracellular multivesicular bodies, secreted in response to upregulation of sphingomyelin phosphodiesterase 3 (SMPD3). The capacity to calcify correlates with the exosome release, which is regulated by pro-calcific stimuli [67]. Interestingly, phosphate-induced autophagy may interfere with the MV release, thereby limiting vascular calcification [68].

j. Oxidative Stress and Inflammation

The effect of chronic inflammation on cardiovascular calcification is not fully understood. Calcification is part of atherosclerosis, which is fundamentally an inflammatory disease, and many inflammatory cytokines are pro-calcific [69,70]. However, media sclerosis is less driven by inflammation. Calcification per se may also enhance inflammation when present in the form of hyperphosphatemia-induced nanocrystals or calcium phosphate crystals [40,71]. It was recently shown that the Singleton-Merten syndrome, which includes both vascular and valvular calcification, might be due to mutations resulting in increased interferon activity [72], thereby supporting the concept that inflammation leads to calcification. It is interesting to note that infection, such as tuberculosis, commonly leads to calcified lesions, a process likely driven by inflammation. However, further studies are needed to understand the intersection between inflammation and vascular calcification.

k. Notch signaling

Notch signaling is well known to have important roles in cell fate determination [73], and has recently been brought into focus in the calcification field. Briot et al. showed that repression of Sox9 by the Notch ligand Jag1 is continuously required to avoid chondrogenesis in vascular SMCs [74]. This extends previous findings on Notch in patients with calcific aortic valve disease, where mutations in the Notch1 receptor caused disruption in the suppression of Runx2 [75].

Conclusions

The expansion of our understanding of vascular calcification has occurred in parallel with progress in vascular and stem cell biology. The various types of vascular calcification, the multiple origins of calcifying vascular cells, and the increasing number of signaling pathways that influence vascular calcification reflect the fundamental plasticity of the vasculature. Our current understanding provides us with numerous possibilities for targeting

vascular calcification in clinical contexts. The challenge is to translate this knowledge into viable preventive and therapeutic strategies.

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Figure 1.

Schematic drawing of different types of vascular calcification affecting elastic arteries in the systemic circulation, including atherosclerotic lesion calcification, calcification of the internal elastic lamina (IEL), coral reef aorta, media sclerosis (Mönckeberg's disease), and porcelain aorta.

Figure 2.

Overview of regulatory systems that influence vascular calcification, as reported in the endothelial, medial and adventitial layers of the vascular wall. BMP, bone morphogenetic protein; FGF23, fibroblast growth factor 23; RAGE, receptor for advanced glycosylation end products; PTEN, phosphatase and tensin homolog; O-GlcNAcylation, O-linked N-acetylglucosamine; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-kappaB ligand.

TABLE 1

Location of Cardiovascular Calcification

Location	Disease	Reference
Systemic arteries: Intima	Atherosclerosis	[4,6]
Systemic arteries: Media	Media sclerosis (Mönckeberg's disease)	[8]
	Porcelain aorta	[12]
	Coral reef aorta	[14]
Internal elastic lamina	Variant of media sclerosis	[9]
Pulmonary arteries	Probable media calcification	[15]
Arterioles	Calcific uremic arteriolopathy (calciphylaxis)	[16]
Cardiac valves	Aortic stenosis, aortic sclerosis	[17,18]
Myocardium	Myocardial calcification	[19,20]

TABLE 2

Potential Sources of Calcifying Cells

Cell Type	Reference
Smooth muscle cells	[22]
Medial non-smooth muscle cells	[23]
Pericytes	[24]
Endothelial cells (endothelial-mesenchymal transitions)	[25,26]
Adventitial myofibroblasts	[27]
Circulating or stationary progenitor cells	[28–30]
Interstitial valve cells	[17]