The Contribution of Osteoprogenitor Cells to Arterial Stiffness and Hypertension

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Key Words

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Abstract

Hypertension, the major cause of cardiovascular disease, is bidirectionally linked to arterial stiffness. Evidence shows that vascular calcification, either medial or intimal, induces arterial stiffening further worsening hypertension parallel to substantially increasing cardiovascular risk. The disturbance in the bone-vascular axis that leads to the increase of calcium deposition in the arterial wall may be the result of a shift in the functionality of bone marrow-derived circulating stem cells with a calcifying potential, namely osteoprogenitor cells. These cells deposit bone matrix proteins in the vascular wall that can subsequently become mineralized. The current notion is that these cells derive from diverse cell lines. The present review summarizes the current knowledge on the role of progenitor cells with a calcifying potential on arterial calcification, stiffness and hypertension.

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Introduction

Osteoprogenitor cells (OPCs) are known to be the progenitors of osteoblasts. They are known to contribute to new bone tissue formation [1] and fracture repair [2]. Current knowledge, however, suggests that not only these

circulating bone marrow-derived stem cells can give rise to cells morphologically and functionally different from osteoblasts, but also that OPCs can derive from different cell types such as monocytes, pericytes and vascular smooth muscle cells (VSMCs) outside the bone microenvironment [3, 4]. All these types of cells, including the ones derived from bone marrow, have been implicated in the formation of ectopic calcification.

The most clinically important site of ectopic calcification is arterial calcification. It is a phenomenon of disturbed calcium deposition, as part of the calcium that is supposed to be deposited in our bones is lodged in our vessels [5]. This pathology of the bone-vascular axis (osteoporosis and vascular calcification, VC) is typically associated with aging and accelerated by age-related diseases such as kidney disease and diabetes.

OPCs, also called circulating calcifying cells, are thought to play a pivotal role in this bone-vascular axis. These cells deposit bone matrix proteins in the vascular wall that can subsequently become mineralized. The ability of OPCs to calcify in vivo is related to the expression of bone-related genes, especially transcription factor Runx-2, a master gene regulator of osteogenesis [6]. Other transcription factors that induce osteogenic expression are Cbfa1, Msx2 and Sox9; these may also affect OPCs to induce arterial calcification [3].

The ectopic arterial calcium deposition is discriminated into medial and intimal and is predictive of cardiovascular morbidity and mortality [7]. There are two forms of VC, each with a distinct anatomical distribution and clin-

Fig. 1. The origin of OPCs. These cells derive from 1) the hemopoietic cell line that gives rise to monocytes and macrophages, 2) mesenchymal cells, 3) perivascular pericytes, 4) VSMCs and 5) stromal cells.

ical relevance, namely the intimal and medial calcification, the latter being more widely known as medial elastocalcinosis or Mockenberg's disease [8]. Intimal calcification involves the lipid-rich fibrous cap of the atheroma and is likely to be the net result of a balance of factors favoring and inhibiting calcification. The former factors include apoptotic cell death of VSMCs and macrophages, calcification stimulatory proteins, lipids and mechanisms which increase the calcium and phosphate extracellular concentration, while the latter factors include phagocytosis, calcification inhibitory proteins and, potentially, osteoclast macrophages [9].

Medial elastocalcinosis is a less frequent phenomenon and characterizes mainly diabetic and chronic kidney disease (CKD) patients. Unlike intimal calcification, medial calcification occurs in the absence of lipids or inflammatory cells. It is likely the result of a lack of expression or activity of calcification inhibitory proteins, degeneration

of elastic fibers, induction of apoptosis and failure of clearance of apoptotic bodies or a de-regulation of the pH [9].

Mounting evidence supports that arterial calcification largely affects arterial stiffness, a well-known consequence of vascular aging and the most important contributor to the development of hypertension and its detrimental consequences, namely stroke and myocardial infarction [10–12].

This review summarizes the direct effects/associations of progenitor cells with a calcifying potential, now known as osteoprogenitors, to arterial stiffening and hypertension.

Origin of OPCs

The current notion on the origin of OPCs is that they derive from diverse cell lines (fig. 1). The concept that there are circulating cells of the osteoblast lineage which

correlate with markers of bone formation came from Eghbali-Fatourehchi et al. [13] in 2005. These cells were thought to be comprised of 2 populations: 1 related to hematopoietic stem cells/endothelial progenitor cells and the other to mesenchymal stem cells [3].

Recently, Fadini et al. [14] showed that there are circulating cells with osteogenic potential that express osteocalcin (OCN+), bone alkaline phosphatase (BAP+) and also the Philadelphia chromosome-related BCR-ABL gene complex in patients with chronic myeloid leukemia, demonstrating a myeloid origin. These originate from the bone marrow, although in the same study they also showed that circulating monocytes/macrophages can transform to myeloid calcifying cells by *in vitro* culture.

Hegyí et al. [15] postulated that OPCs originate from perivascular pericytes, medial VSMCs, adventitial fibroblasts or from the bone marrow stroma, highlighting the strong association between angiogenesis and heterotopic ossification of the skeletal muscle. Pericytes that are present in the new blood vessels may become OPGs, suggesting that these cells may provide the cellular link between ectopic calcification and angiogenesis [16].

Another potential origin of OPCs is the dedifferentiation of artery wall cells into mesenchymal cells such as osteoblasts, the presence of cells with osteoblastic dedifferentiation within the vessel wall [17, 18]. These cells are likely to be VSMCs. In particular, VSMCs acquire an osteogenic phenotype that is activated by the activation of the elastin and transforming growth factor- β receptors by the respective molecules that are produced by matrix degradation through the elastolytic matrix metalloproteinases [19]. In a recent study, mesenchymal cells enhanced their osteoblastic differentiation by the combined action of bone morphogenetic proteins (BMP) and vascular endothelial growth factor [20].

It also seems that inflammatory cytokines such as TNF- α induce elongated VSMCs to transform into a cuboidal cell, which is highly reminiscent of an osteoblast. TNF- α increases calcium deposition and the expression of osteogenic signals like alkaline phosphatase and BMP-2 [21].

OPGs may increase in number in states of high BMP activity, such as when there is a lack of the BMP inhibitor matrix Gla protein (MGP) and hyperglycemia. In this case, an animal study showed that the osteogenic cells are derived from the vascular endothelium. The expression of BMP-2 may be regulated by an H₂O₂-mediated activation of NF- κ B both by inflammatory stimuli and by high intravascular pressure [22]. VC could, therefore, be considered an acquired stem cell disorder in these settings [23, 24].

A different origin of the OPCs has been proposed depending on whether the calcification is medial or intimal. Speer et al. [25] investigated the origin of cells involved in medial calcification and showed that they derive from smooth muscle cells and not from bone marrow cells. Additionally, Doerhing et al. [26] showed that there are a number of cells in the intimal lesions that are CD34+ and bone marrow-derived.

The induction signals for this acquired osteogenic potential in either bone marrow-derived cells or VSMCs likely comes from the same regulators, namely the BMP/Smad6 signaling pathways. In this way, the expression of the BMP pathway is highly dependent on the expression of the transcription regulator Runx. On the other hand, loss of inhibitors of VC such as fetuin A or MGP-1 induces matrix vesicle formation and VSMC-induced calcification [26].

A common substrate of osteoporosis and arterial stiffening could be inflammation, which drives bone resorption and promotes vascular disease [27]. Novel research by Al-Aly [21] showed that inflammation has a central role in the generation of vascular cells that calcify *in vivo*. In this context, the chief inflammatory mediator TNF- α dose-dependently increased Msx2 mRNA and BMP2 in aortic mural progenitor cells in high-fat fed mice with diabetes.

Arterial Stiffness and Bone Mineral Density

In the Baltimore Longitudinal Study on Aging (BLSA) [28], there was a significant reverse association between pulse wave velocity (PWV), a reliable surrogate marker of cardiovascular risk, and cross-sectional cortical bone area in women, but not in men, after adjusting for confounders. This finding suggests that mediators of this association probably are differentially regulated between men and women. In this study, in women, every SD decrease in cross-sectional cortical bone area was associated with a 0.14 SD increase in PWV, an effect quite modest, given that age is the major determinant of PWV [28].

Four other studies investigating the association between bone demineralization and arterial stiffness in women also found an inverse relationship between these two variables [29–32]. These studies were all done in osteoporotic women and used dual energy X-ray absorptiometry scans, and all of them showed a greater increase in PWV with every decrease in lumbar spine bone mineral density than the BLSA study. The higher effect size of these studies is probably due to the patient selection, given that the former studies included osteoporotic patients.

In 2003, Hirose et al. [33] showed that carotid-femoral PWV significantly correlated with the osteo-sono assessment index – an index of bone strength assessed by quantitative ultrasound – in both genders, with a more prominent relationship in females, especially those in menopause. It seems that a common denominator for these observations is the rapid estrogen decrease in early postmenopause and that this can be partially reversed after estrogen replacement. Zaydun et al. [34] provided relevant data suggesting that the age-related increase in arterial stiffness in early postmenopausal phase is at least in part related to estrogen deficiency. Tanaka et al. [35] showed by computed tomography that in patients with rheumatoid arthritis the bone loss was significantly associated with arterial stiffness.

These studies may indicate a possible role in cells that regulate both periarticular bone loss and arterial stiffness and are involved in the bone-vascular axis, such as osteoblasts and their progenitors.

Arterial Calcification, Arterial Stiffness and Hypertension

We know by now that VC is associated with hypertension and that the onset of arterial calcification occurs at an earlier stage in hypertensive subjects [36]. In these patients, coronary artery calcification seems to predict long-term mortality as shown by a substudy of the INSIGHT trial [7]. Moreover, it has been demonstrated that coronary artery calcification is associated with an increased occurrence of ischemic stroke in hypertensives [7, 37].

The mechanism behind this observation is that arterial calcification causes a progressive reduction in vascular resilience and compliance with a parallel increase in arterial stiffness, which is a major determinant of the rise in systolic blood pressure (BP), the fall in diastolic BP and the acceleration of PWV.

This phenomenon is very prominent in the well-known animal model of VC MGP (–/–) mice. These are knockout mice for MGP, a potent matrix mineralization inhibitor. They are known to be tachycardic with a very high PWV in the aorta at rest, presumably from the dramatic increase in aortic calcium and endothelial dysfunction as evidenced by the minimal inflammatory response to hyperlipidemia and the formation of arteriovenous malformations [38]. These hemodynamic alterations increase the left ventricular load and susceptibility to myocardial ischemia [3].

Several studies proved an association between carotid-femoral PWV and arterial calcification in both animal

models and humans (tables 1, 2). In CKD, where medial calcification is prominent, indirect evidence in humans showed the calcification inhibitor fetuin-A and the anti-osteoclastic factor osteoprotegerin to be independently associated with arterial stiffness [39].

Moreover, in an early study [40] in patients with end-stage renal disease, increased VC was associated with increased stiffness of large-capacity elastic-type arteries like the aorta and the common carotid artery. Later, two other human studies verified this association [41, 42] (table 2).

Outside of CKD, direct evidence of the VC and arterial stiffness comes from two studies in mice. In the first, medial elastocalcinosis induced by hypervitaminosis D plus nicotine, demonstrated a strong relationship between the extent of aortic calcification and stiffness measured by several approaches [43]. Essalihi et al. [44] confirmed this data in a warfarin-induced medial elastocalcinosis rat model. The authors concluded that at least one third of the stiffness was related to elastocalcinosis.

In asymptomatic humans, Seo et al. [31] showed that aortic calcification was significantly correlated with carotid-femoral PWV in a study of 193 drug-naïve subjects after adjusting for confounders. In accordance with this, McEniery et al. found a significant association between aortic stiffness and isolated systolic BP [45]. Moreover, in the twin UK study, a significant correlation was found between VC and PWV in a subgroup of 40 women [46]. The largest study ever to assess this relationship was the Multi-Ethnic Study of Atherosclerosis (MESA), in which aortic calcification significantly correlated with carotid arterial stiffness [47].

The role of OPCs in this relationship has been described in an in vitro study where in rats with CKD and VC, smooth muscle cell differentiation to osteoblast-like cell was associated with medial remodeling, which included disruption of the elastic lamellas and deposition of collagen, which was at least in part responsible for the observed arterial stiffness [48].

It is noteworthy that the translation of the rise in PWV velocity to rise in BP is not parallel, and while the two are interdependent, the rates of their acceleration differ across age and gender. This has been recently demonstrated in two major studies that dissociate PWV from BP trajectories. Specifically, in the BLSA, the rates of PWV increase accelerated with advancing age in men more than in women, leading to gender differences in PWV after the age of 50 [49].

Likewise, in the SardiNIA study, at ages >40 years in men, the rates of change in systolic BP and pulse pressure

Table 1. Animal studies of the effects of induced VC on arterial stiffness

Authors [Ref.], year	Subject characteristics	Number	Methodology, measurements	Results
Luo et al. [38], 1997	Mice lacking the matrix Gla protein	11 mice	PWV	Increased PWV and tachycardia. Mice develop to term but die within 2 months as a result of arterial calcification which leads to blood vessel rupture
Niederhoffer et al. [43], 1997	2-month-old male rats, injected with VDN, and controls	21 VDN 14 controls	Tail systolic arterial BP and heart rate, aortic BP, PWV, pulse amplification and wave reflections. Aortic wall composition, aortic structure and elastic modulus, wall stress and cardiac mass were measured at baseline and 2 months after treatment	The VDN rats developed ISH. No difference in aortic wall thickness and internal diameter between 2 groups. Wall stiffness increased after VDN and was significantly correlated to the wall content of calcium. Left ventricular hypertrophy occurred in the VDN model
Essalihi et al. [44], 2003	Rats treated with WVK for 4 and 8 weeks	14 WVK (4 weeks) 8 controls (4 weeks) 8 WVKs (8 weeks) 10 controls (8 weeks)	PP, PWV, left ventricular hypertrophy	WVK treatment caused medial calcification, proportional elevation of PP, increased collagen and decreased elastin in the aorta, and increased PWV. Left ventricular and aortic hypertrophy remained normal
ISH = isolated systolic hypertension; PP = pulse pressure; VDN = vitamin D ₃ and nicotine; WVK = warfarin and vitamin K.				

increase plateaued and then declined, so that the systolic BP itself also declined at older ages, whereas the pulse pressure plateaued [50]. This dissociation may be due to different ways that calcification occurs across age and gender, thus affecting the BP phenotype.

Osteoprogenitors Arterial Stiffness and Cardiovascular Disease

In 2010, Pirro et al. [51] found for the first time that osteoporotic women had a significantly higher absolute number of OPCs (defined as CD34+/AP+ or CD34+/OCN+) than nonosteoporotic controls, while in the regression analysis, CD34+/OCN+ were significant predictors of aortic PWV, along with systolic BP, heart rate and age after adjusting for CV risk factors, parathyroid hormone levels and osteoporotic status. In this study, osteoporotic women also had a higher PWV compared to controls. The same research group showed that osteoporotic women with abdominal aortic calcium deposition had a higher number of circulating CD15-/AP+/OCN- cells [52].

Mangiafico et al. [53] showed that osteoporotic women had higher PWV, augmentation index and central aor-

tic and pulse pressures compared to controls. Pal et al. [54] showed that in patients with peripheral artery disease, circulating osteocalcin-positive mononuclear cells (OCN+ MNCs) were associated with the severity of aortic calcification. In 2010, Pirro et al. [51] demonstrated the CD34+/OCN+ cell population as the cell lineage whose excess is closely related to bone mineral density and arterial stiffening. Gossel et al. [55] found a greater percentage of CD34+/KDR+ cells in subjects with early coronary atherosclerosis, characterized by coronary endothelial dysfunction, or in subjects with severe multivessel coronary artery disease compared to those with no structural coronary artery disease.

Future Perspectives

Despite their origin, OPCs are involved in the extraosseous calcification in vessels, which is a result of the dysregulation of promoters and inhibitors of VC and may be the result of increased oxidative stress [56]. This ectopic calcification, whether it is intimal or medial, results in arterial stiffness, with its detrimental effects on arterial age.

Table 2. Human observational studies correlating arterial stiffness with VC

Authors [Ref.], year	Subject characteristics	Number	Methodology	Correlation
Guerin et al. [40], 2000	ESRD patients on hemodialysis stable for >1 year	120	Aortic PWV and calcium score 0–4 in the common carotid artery measured by ultrasonography	Regression analysis β -coefficient = 52.85, $R^2 = 0.54$, $p < 0.001$, after adjusting for age and MAP
Toussaint et al. [42], 2007	CKD (17–55 ml/min), diabetic and nondiabetic	48	PWV measured by SphygmoCor. CT of the abdominal aorta and superficial femoral arteries	Univariate linear regression analysis $r = 0.33$, $p = 0.03$
Temmar et al. [41], 2010	CKD stages 2–5	150	PWV measured by SphygmoCor. Multislice spiral CT scan for aortic calcium and CAC score measurement	Multivariate regression analysis β -coefficient = 0.197 for the correlation of PWV and aortic calcification, $R^2 = 0.487$. The model included the MAP, age and the presence of diabetes as covariates
Cecelja et al. [46], 2011	UK twin cohort, a cohort with characteristics similar to the UK general population	900	PWV measured by SphygmoCor. Noncontrast-enhanced helical CT scan for carotid and aortic calcification	C-f PWV correlated with the calcified atheroma, β -coefficient = 0.08. The model included age, MAP, heart rate and glucose
Seo et al. [31], 2009	Postmenopausal women	152	B-a PWV and 64-row multidetector CT	Multivariate logistic regression B-a PWV significantly influenced the presence of coronary atherosclerosis. OR = 11.7 (95% CIs 1.39–99.5) after adjusting for confounders
Blaha et al. [47], 2009	Asymptomatic men and women	6,814	Distensibility coefficient using high-resolution B-mode ultrasonography of the right and left carotid. Thoracic aortic calcification using cardiac gated electron beam CT	Individuals in the stiffer quartile had a prevalence ratio of 1.52 (95% CI 1.15–2.00) for thoracic aorta calcification compared with the least stiff quartile after adjusting for confounders

B-a = Brachial-ankle; CT = computed tomography; ESRD = end-stage renal disease; C-f = carotid-femoral; MAP = mean arterial pressure; CAC = coronary arterial calcification.

The role of OPCs in osteoporosis was directly shown by Pirro et al. [57] who found a significantly higher number of AP+/CD34+ and OCN+ in osteoporotic postmenopausal women compared to controls, and the number of CD34+/OCN was associated with reduced bone mineral density.

Based on this observation, Peris et al. [58] investigated the effect of osteoporosis treatment on the progenitor cellular level. This study showed that treatment with risendronate resulted in the downregulation of osteogenic genes in endothelial progenitor cells and a trend of a reduction of endothelial progenitor cells with osteogenic potential (those coexpressing osteoblastic surface markers and genes). These data may indicate a possible mechanism by which bisphosphonates may inhibit VC.

Other possible treatments that may have an effect on the OPC number are monoclonal antibodies such as denosumab, an anti-RANKL ligand that significantly attenuated aortic calcium deposition in human RANKL knock-in mice with glucocorticoid-induced osteoporosis [59]. However, in humans, denosumab did not influence the 3-year progression of aortic calcification or the incidence of adverse cardiovascular events in postmenopausal women with osteoporosis and high cardiovascular risk [60].

Another possible monoclonal treatment to intervene in the bone-vascular axis is the new drug romosozumab which targets sclerostin. Sclerostin levels are found to be positively associated with both aortic calcification and arterial stiffness in postmenopausal women [61]. They are also found to be strongly associated with calcifying aortic

heart valve disease in hemodialysis patients [62]. However, increased sclerostin levels have been associated with improved survival in hemodialysis patients [63].

These aforementioned treatments may provide a unifying approach to the now well-recognized dysregulated bone-vascular axis and disrupted OPC number in age-related diseases. To what extent these drugs may provide collateral benefits beyond improving osteoporosis, such as improving VC and stiffness, is yet to be investigated.

Other drugs that may interfere with the bone-vascular axis include tumor-suppressing agents which are inhibitors of the canonical signaling Wnt pathway, which is actively involved in bone formation and VC, such as secreted frizzled-related proteins 2 and 4 and the Dkkopf-related protein 1. However, especially for secreted frizzled-related proteins, which are circulating secreted proteins, they act systematically and may reduce bone mass [64].

Several antihypertensive drug classes have also been shown to reduce VC [65]. These agents, which include drugs that interfere with the renin-angiotensin axis as well as calcium channel blockers may decrease calcification of VSMCs, thus inhibiting the adoption of an osteogenic phenotype. However, this remains to be investigated.

It is not yet clear whether lowering vascular calcium deposits is beneficial in the long term. The exact implications of VC on plaque stability are still under debate; however, since the distribution of calcification rather than its mere presence may predispose to rupture, plaques with

diffused and speckled microcalcium deposits ('spotty calcification') are more vulnerable [66]. In this context, whether therapies targeting OPCs produce favorable outcomes can only be speculated.

Investigating potential targets for treatment at the progenitor cellular level is a novel approach to long-standing debilitating problems such as osteoporosis and cardiovascular disease. Whether killing two birds with one stone is feasible using known treatments remains a challenge for future research.

Conclusions

There is mounting evidence of a role for OPCs in VC. These cells derive from diverse cell lines. Loss of inhibitors and induction of promoters of VC may lead cells that derive from pericytes, VSMCs, monocytes or stromal cells to acquire an osteogenic phenotype. OPCs are thought to induce both intimal and medial calcification, thus promoting arterial stiffness, hypertension and cardiovascular disease. Several treatments for osteoporosis, such as biphosphonates or the anti-RANKL ligand as well as antihypertensive medication, may influence OPC number and VC.

Disclosure Statement

The authors declare no conflict of interest.

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