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The Bone-Vascular Axis: A Key Player in Chronic Kidney Disease Associated Vascular Calcification

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Keywords
Bone-vascular axis · Bone metabolism marker · Chronic kidney disease · Vascular calcification · Osteoporosis

Abstract
Background: The bone-vascular axis plays a key role in the pathogenesis of vascular calcification (VC) in patients with chronic kidney disease (CKD). Understanding and managing the role of the bone-vascular axis in CKD-mineral and bone disorder (CKD-MBD) is critical for preventing and treating associated complications, including osteoporosis, arterial calcification, and cardiovascular diseases. This study aimed to comprehensively summarize the role of bone metabolism markers in uremic VC. ***Summary:*** The skeleton, as an endocrine organ, can regulate systemic metabolic processes by secreting various bioactive substances. These molecules can induce the transdifferentiation of vascular smooth muscle cells, promoting their transition to other functional states, thereby affecting vascular growth and remodeling. ***Key Messages:*** The prevalence of VC in individuals with CKD is notably high. CKD-associated VC is characterized by the widespread accumulation of hydroxyapatite within the arterial media, which occurs as a result of the transformation of smooth muscle cells into osteoblastic smooth muscle cells under the influence of uremic toxins. Osteoblasts and osteoclasts in bone tissue secrete mineral metabolic proteins, which can influence neighboring cells, primarily vascular smooth muscle cells, through paracrine signaling. Both circulating and osteocytic sclerostin can exert a protective effect by inhibiting wingless/integrated (WNT)-induced calcification. The therapeutic goal for CKD-MBD is to reduce production of sclerostin by decreasing the osteogenic transdifferentiation of vascular smooth muscle cells. Calciprotein particles act as a physiological agent for delivering calcium-phosphate the bone and inducing fibroblast growth factor-23 expression in osteoblasts.

Plain Language Summary
The bone-vascular axis is crucial in the pathogenesis of VC in CKD, impacting mineral and bone disorders and leading to complications like osteoporosis and cardiovascular diseases. This study focuses on the role of bone metabolism markers in uremic VC. The skeleton functions as an endocrine organ, releasing bioactive substances that facilitate the transdifferentiation of vascular smooth muscle cells (VSMCs) into osteoblastic cells, which contributes to vascular remodeling and calcification. Notably, circulating and osteocytic sclerostin can protect against calcification by inhibiting WNT signaling pathways. Therefore, a therapeutic goal for CKD-

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related mineral and bone disorder (CKD-MBD) is to lower sclerostin levels to reduce VSMC transdifferentiation. Additionally, calciprotein particles play a role in regulating calcium-phosphate transport to bones and stimulating fibroblast growth factor-23 expression in osteoblasts. Understanding these mechanisms is essential for developing effective strategies to manage VC in CKD patients.

Introduction

Chronic kidney disease (CKD) associated vascular calcification (VC) focusing on uremic toxin induced calcification of vascular or other soft tissues, is one vital part of CKD-mineral and bone disorder (CKD-MBD), besides hyperphosphatemia, hyperparathyroidism and abnormalities in bone turnover, mineralization, volume [1].The 2006 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines suggest that lateral abdominal radiography can be used to assess the extent of aortic valve calcification, serving as a semi-quantitative approach. This is a simple and cost-effective screening method for adult VC [1]. In 2009, KDIGO updated its guidelines (available from: http://www.kidney-international.org) and dedicated a separate chapter to VC. The diagnostic criteria for CKD-MBD include the identification of ectopic calcifications, such as arterial, valvular, and myocardial calcifications. The most sensitive methods for detecting and quantifying cardiovascular calcification (CVC) are electron beam computed tomography and multi-slice spiral computed tomography. While the CT-based coronary artery calcification score is widely regarded as the gold standard for assessing CVC in patients with CKD and the general population, other simpler and more convenient methods, such as lateral abdominal X-rays, pulse wave velocity measurement, and echocardiography, are available. Echocardiography can be used to detect valvular calcification in patients with CKD stages 3–5. The 2017 KDIGO guidelines (available from: http://www.kidney-international.org) include almost no updates or revisions to the VC section [2, 3].

Clinical Understanding of CKD-Associated VC

Epidemiology

In patients with CKD, VC refers to the abnormal deposition of calcium-phosphate salts in the blood vessel walls and heart valves. This phenomenon can occur in the coronary, abdominal aorta, iliac, and femoral arteries. CVC is a recognized component of CKD-MBD. Epidemiological studies suggest that ischemic heart disease, sudden cardiac death, arrhythmias, heart failure, stroke, and peripheral arterial disease together account for >50% of late-stage CKD-related deaths [4]. Patients with CKD and CVC have a higher risk of mortality due to cardiovascular causes [5]. For patients on dialysis, the annual incidence of aortic valve calcification is approximately 3.3%, while the prevalence of calcification in the aortic and mitral valves is 25–59% [6]. Furthermore, the prevalence of VC in patients undergoing hemodialysis (HD) is eight times higher than that in the general population [6]. Given these heightened risks, it is imperative to comprehensively understand the pathophysiology of CKD-MBD and its effects on the cardiovascular system to prevent cardiovascular diseases and improve the prognosis of patients with CKD.

CAC is a strong biomarker of high cardiovascular risk in patients with CKD, particularly those undergoing regular dialysis [7]. In recent years, growing evidence has shown that mineral and bone metabolism disorders are associated with an increased risk of CVC, morbidity, and mortality. Although the exact underlying mechanisms remain unclear, the involvement of changes in VC that impact cardiovascular structure and function has been suggested. Therefore, the evaluation of ectopic calcification has become an indispensable component in the diagnosis and classification of mineral and bone disorders in patients with CKD-MBD.

Characteristics of VC in CKD-MBD

The blood vessels were comprised of three layers. The inner layer, known as the tunica intima, predominantly consists of endothelial cells (ECs) that respond to circulating factors. These cells regulate vascular permeability and govern various processes, including blood coagulation, fibrinolysis, and platelet adhesion. The middle layer, termed the tunica media, primarily comprises smooth muscle cells that are responsible for vessel contraction and dilation. The outer layer, called tunica adventitia, is composed of connective tissue and fibroblasts [8]. Arterial calcification can be classified into three main types, each with distinct characteristics. The first type, intimal calcification, tends to be localized and is typically associated with atherosclerosis, where lipid deposits and inflammatory infiltrates damage ECs [9, 10]. This type of

Table 1. Comparison between intimal and medial VC

	Intimal calcification	Medial calcification
Distribution	Focal	Diffusive
Mechanism	Associated with lipid deposition and inflammatory infiltration	Can occur without lipid deposition or immune cell infiltration
Deposits	Cholesterol	Hydroxyapatite crystals
Type of ossification	Endochondral	Intramembranous
Pathogenic mechanism	Atherosclerosis, plaque rupture	Arteriosclerosis, increased cardiac afterload
Risk factors	Hypertension, diabetes, hypercholesterolemia, smoking	More prevalent in patients with CKD

calcification resembles endochondral ossification, involving characteristic clusters of osteoblasts and chondrocytes that gradually mineralize cartilage matrix precursors or anlagen [11]. Its occurrence and progression are driven by genetic and lifestyle factors, such as hypertension, diabetes, hypercholesterolemia, and smoking [12]. Atherosclerotic intimal calcification is associated with arterial lumen narrowing and can lead to plaque rupture. Intimal calcification reflects the overall burden of atherosclerotic plaques and serves as a strong predictor of cardiovascular events and mortality [13, 14].

Alternatively, medial calcification can occur independently or concurrently with atherosclerosis. Initially referred to as "Monckeberg's sclerosis," this type of calcification resembles intramembranous ossification, originating from the mineralizing activity of osteoblasts without chondrocyte involvement [11]. Medial calcification can cause arterial stiffness, increased pulse pressure, and elevated pulse wave velocity, thereby leading to left ventricular hypertrophy, dysfunction, and failure [9]. Furthermore, extensive calcification in the heart valves can contribute to heart failure and increase the risk of endocarditis. In dialysis patients, medial calcification is closely related to the duration of HD and imbalances in calcium-phosphate metabolism [13]. In addition, it is associated with increased cardiac afterload and tends to have a more diffuse distribution, often occurring without lipid deposition or immune cell infiltration [9, 14]. This type of calcification predominantly affects arteries that are less susceptible to atherosclerosis, including visceral abdominal, thyroid, pulmonary, limb, and femoral arteries, a pattern more typical in patients with CKD-MBD [15]. A recent study focusing on patients with CKD stage 5D found that VC is common in large arteries such as the aorta (approximately 80%), medium-sized arteries such as the coronary arteries (60%–70%), and smaller arteries (20–30%) [12]. Previous research suggests that VC is a passive process caused by the combined effects of cell death and infiltration of hydroxyapatite minerals into the blood vessels [16]. However, recent studies have shown that VC is an active process that resembles bone formation, primarily driven by the phenotypic transformation of vascular smooth muscle cells (VSMCs). During this process, smooth muscle cells convert from their typical spindle shape to a spherical osteoblast-like form, which induces biomineralization of the extracellular matrix (ECM). Calcium-phosphate nanocrystals are composed of calciprotein particles (CPPs), mineralized extracellular vesicles (EVs), and mineral deposits in the vascular ECM [6, 13]. CPPs are nanoparticles composed of calcium, phosphate, and serum proteins, including fetuin-A, albumin, and other acidic proteins [6]. Table 1 shows the differences between the two types of VC.

Calciphylaxis, also referred to as calcific uremic arteriolopathy, is a rare but extremely severe type of calcification in the intima and media layers of small arteries. This condition can be life-threatening and typically presents as thrombotic occlusion, tissue ischemia, and necrosis [17].

Bone-Vascular Axis and Related Factors in CKD-MBD

The coexistence of bone loss with progressive VC should be considered a paradoxical phenomenon since calcium is reduced in the bones but accumulates in the

blood vessels in the opposite direction. This is commonly referred to as "calcification paradox" [18]. Another important concept that connects osteoporosis and VC is the bone-vascular axis. Previously, the understanding of the bone-vascular axis was limited to a superficial perspective, merely referring to the interconnection and mutual influence between the skeletal system and the cardiovascular system. Nowadays, it is assumed that common pathophysiological processes are located in both pathological VC and osteoporosis, as if an invisible hand is transferring calcium from the bones to the walls of blood vessels [19]. The nature of these links is not well understood.

Embryologically, VSMCs and osteoblasts originate from the same mesenchymal stem cell lineage, and the interaction between osteogenic and vascular factors contributes to the coordinated development of bones and blood vessels [20]. A clinical study conducted in 2022 found that aortic calcification and high pulse wave ankle-brachial index were negatively correlated with bone mineral density (BMD) in patients with CKD [21]. Additionally, reduced BMD in the femur and femoral neck, along with total BMD, were identified as risk factors for valvular heart disease [21]. In patients with CKD, bone damage primarily presents as chronic trabecular bone loss, with more severe VC positively correlating with greater cancellous bone loss [22]. Concurrently, VC and osteoporosis frequently coexist in these patients, and both are considered mutually equivalent in terms of risk. Additionally, any treatment affects bone metabolism can directly or indirectly affect vascular health. Therefore, clinicians should closely monitor the relationship between osteoporosis and VC when managing CKD patients [23].

Various markers related to bone metabolism have been identified in the blood of CKD patients, including osteoprotegerin (OPG) [24], osteopontin (OPN), osteocalcin [25], bone morphogenetic protein (BMP)-2 [11, 26], bone-specific alkaline phosphatase (B-ALP) [27], and runt-related transcription factor 2 (RUNX2) [28]. The intricate interplay between these markers forms a regulatory network that governs the function of VSMCs. These factors play crucial roles in preserving vascular health and in regulating VC.

Fibroblast Growth Factor-23 and Klotho

Fibroblast growth factor-23 (FGF23) is a recently discovered endocrine product, with implications not only in bone diseases but also in kidney and parathyroid metabolism. It serves as a biomarker and plays a key role in kidney diseases [29]. Klotho is a transmembrane protein that acts as a co-receptor to enhance the affinity and specificity of FGF binding to fibroblast growth factor receptors, facilitating FGF23-mediated receptor activation. FGF23 exerts its effects on target organs by binding to a heterodimeric complex of fibroblast growth factor receptors and α-Klotho (Klotho/αK1) co-receptors, forming a 1:1:1 ternary complex [29].

FGF23 and Klotho are hormones that play a crucial role in the metabolic axis of osteovascular metabolism in CKD. FGF23 is negatively correlated with Trabecular Bone Score (TBS), while klotho is positively correlated with TBS. FGF23 and klotho, in combination with TBS, show promise as early markers of trabecular bone impairment in CKD [30]. Among HD patients, higher levels of serum intact FGF23, rather than C-terminal FGF23, were associated BMD values at the lumbar spine and femoral neck [31]. However, further research is needed to validate these findings.

Increased FGF23 plasma levels are associated with declining kidney function and serve as a predictor for future cardiovascular mortality risk [32]. In addition, low Klotho/FGF23 ratio was significantly associated with increased renal events in the cohort of Korean predialysis CKD patients [33]. Furthermore, a meta-analysis was conducted to elucidate the role of klotho and FGF23 in human arterial remodeling across recent studies, specifically focusing on arterial calcification, thickness, and stiffness [34]. Concretely speaking, FGF23 primarily induces left ventricular hypertrophy and heart failure, while klotho deficiency primarily contributes to arterial calcification and atherosclerotic disease combined with hyperphosphatemia [35].

Bone Marrow Mesenchymal Stem Cell Exosomes

In 2022, Wang et al. published a compelling perspective in "Nature Communications," suggesting that EVs derived from an aging bone matrix could serve as conveyors of the calcification paradox [36]. During bone resorption, osteoblast-secreted EVs are released from the aging bone matrix into the bone marrow [36]. These vesicles facilitate the expression of peroxisome proliferator-activated receptor gamma and adipogenic differentiation of bone marrow mesenchymal stem cell (BMMSC) by delivering miR-483-5p, resulting in an imbalance between bone and fat, leading to osteoporosis [36]. Simultaneously, the same vesicles originating from the aging bone matrix can traverse the circulatory system

and accumulate in the blood vessels. Through the transfer of miR-2861, they induce the expression of RUNX2 in VSMCs and drive their transition into osteoblast-like cells, ultimately culminating in VC. This mechanism offers an insight into the calcification paradox [37].

Recent studies have indicated that bone marrow mesenchymal stem cells can secrete exosomes (Exos). However, there have been limited investigations into the effects of BMMSC-Exos on VC within the CKD field. Previous research has demonstrated that BMMSC-Exos can inhibit vascular smooth muscle calcification, although the precise mechanisms underlying this phenomenon remain elusive. Liu et al. [38] conducted a series of studies on BMMSC-Exos in 2019 and found that BMMSC-Exos could reduce high phosphate-induced calcification in human aortic VSMCs by regulating the miRNA profile [38]. In 2021, they further elucidated that BMMSC-Exos could hinder high phosphate-induced transdifferentiation and calcification of VSMCs by targeting the NONHSAT 084969.2/NF-κB axis [39]. Additionally, miR-381-3p, which directly targets NFAT5 mRNA, plays a significant role in this process [40]. Moreover, BMMSC-Exos inhibited high phosphate-induced aortic calcification and ameliorated renal function through the SIRT6-HMGB1 deacetylation pathway [41].

Sclerostin and the Wingless/Integrated Signaling Pathway

The wingless/integrated (WNT) signaling pathway seems to exert a significant influence on VC. Multiple studies indicate markedly higher serum sclerostin levels in patients with CKD compared to those without CKD [42, 43]. This elevation in sclerostin levels in CKD is attributed to increased production rather than impaired kidney function, which affects its excretion [44]. In theory, dedifferentiated VSMCs can transdifferentiate into osteoblasts/chondrocytes or adipocytes [45]. The phenotypic fate of these cells depends on extracellular stimuli, which can activate or inhibit various signaling pathways [45]. Differentiation into osteoblasts and chondrocytes is governed by the Wnt/β-catenin signaling pathway [46]. Conversely, peroxisome proliferator-activated receptor gamma signaling pathway is a strong inducer of adipocyte differentiation [45–47], as shown in Figure 1.

Researchers have identified several interesting patterns. Given the similarity between the development of ectopic VC and bone formation, it is reasonable to speculate that sclerostin may play a role in this pathological process by promoting ectopic calcification by stimulating osteogenic activity in arterial vessels. However, studies involving mice with adenine-induced renal damage have shown that sclerostin gene knockout results in broader VC, indicating that sclerostin may have a protective role in VC [48]. Sclerostin secreted by calcified blood vessels may provide some protection against VC but can also worsen bone disease simultaneously [49]. Sclerostin from different sources may have varying effects on the bone-vascular axis in CKD-MBD. On one hand, sclerostin may be involved in the vascular influence on bone. Most experts believe that elevated sclerostin levels in the serum mainly originate from calcified smooth muscle cells [47]. Research has shown that calcified aortas from uremic rats secrete large quantities of sclerostin and Dkk1 when cultured outside the body. When incubated with osteoblast-like UMR-106 cells, these calcified aortic rings strongly inhibit the formation of calcium crystals [50]. On the other hand, sclerostin also participates in the influence of the bone on blood vessels. In patients with CKD, the 1α-hydroxylase enzyme is deactivated in the kidneys. Damaged kidneys stimulate 1α-hydroxylase activity in bone cells, leading to an increased local production of 1,25(OH)2D through elevated cytokine release. This, in turn, reduces BMP-2 in bones and stimulates the production of osteocytic sclerostin. Osteocytic sclerostin can inhibit BMP-2-induced osteogenic transdifferentiation in arterial walls, thereby alleviating arterial calcification [51]. In summary, both circulating and osteocytic sclerostin can exert a protective effect by inhibiting WNT-induced calcification. This finding helps elucidate why anti-sclerostin monoclonal antibodies, which block sclerostin activity, can enhance bone remodeling, but may also promote VC [52]. Therefore, the treatment objective for CKD-MBD should not solely focus on antagonizing sclerostin activity, but rather on reducing sclerostin production by minimizing osteogenic transdifferentiation in VSMCs [10]. As graphically shown in Figure 2, the role of Sclerostin in CKD-MBD is clearly illustrated.

OPG and the Receptor Activator of Nuclear Factor κ-B Ligand Signaling Pathway

Osteoprotegerin is a glycoprotein produced by osteoblasts and is a member of the tumor necrosis factor superfamily. It regulates bone homeostasis primarily through the receptor activator of nuclear factor κ-B ligand (RANKL) signaling pathway, helping maintain bone

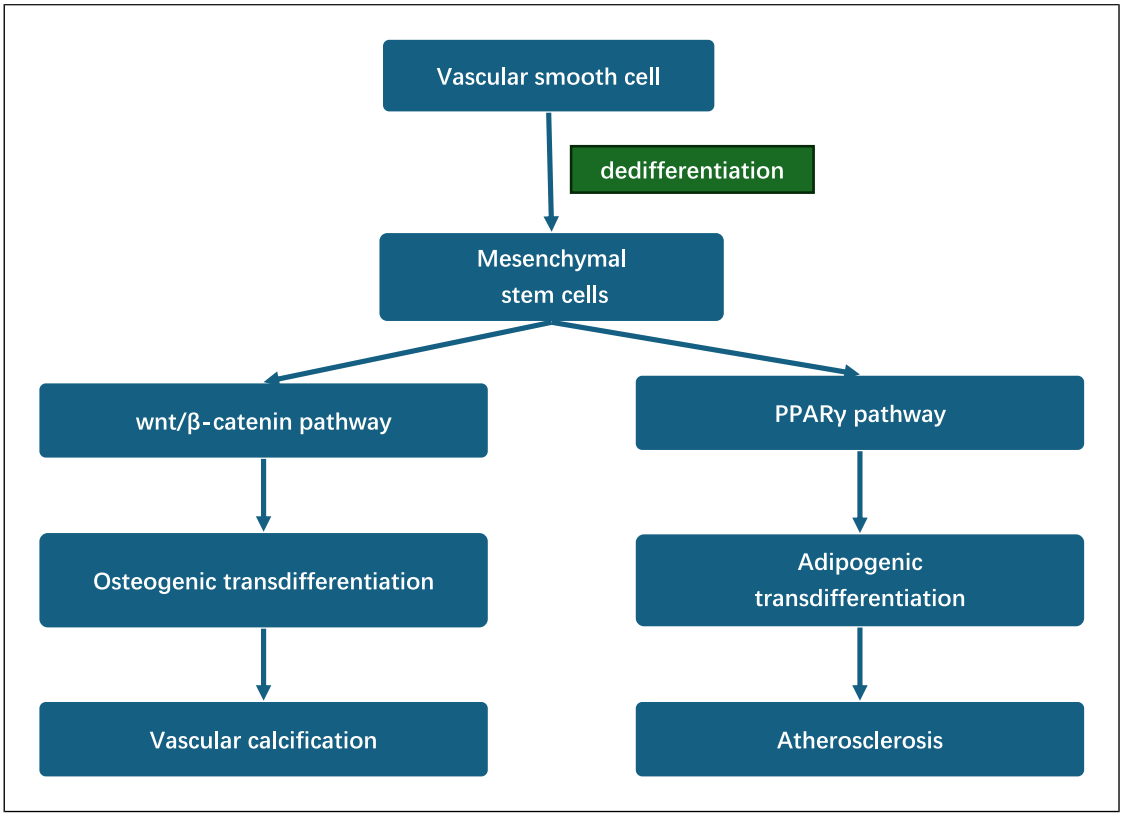

Fig. 1. Opposed interplay between the canonical Wnt/β-catenin pathway and PPARγ pathway in vascular smooth muscle cells Dedifferentiated VSMCs can transform into osteoblasts/chondrocytes or adipocytes depending on extracellular stimuli that activate or inhibit signaling pathways. The Wnt/β-catenin pathway governs differentiation into osteoblasts and chondrocytes, while the PPARγ pathway strongly induces adipocyte differentiation. PPARγ, peroxisome proliferator-activated receptor gamma.

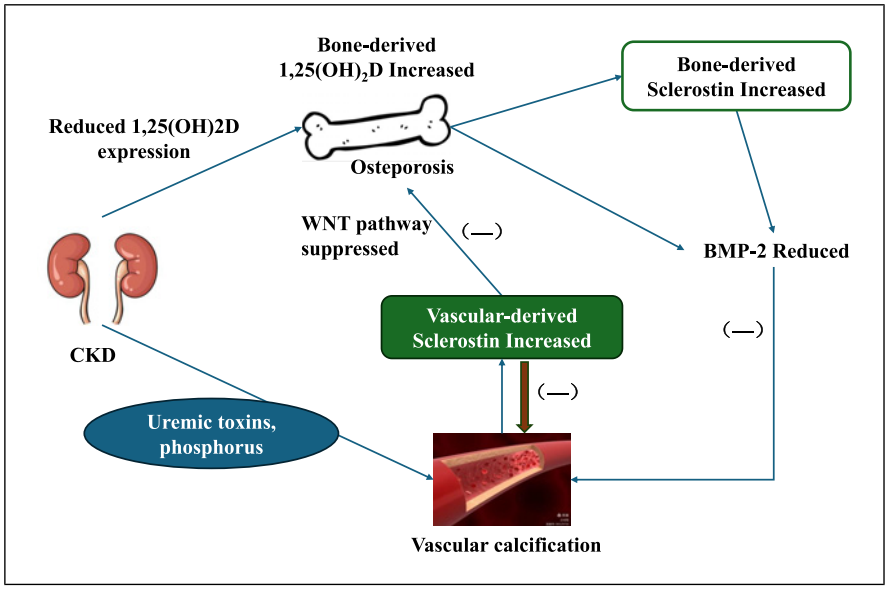

Fig. 2. Role of sclerostin in CKD-MBD sclerostin mediates the intricate interactions between bone metabolism and vascular health, affecting both bone formation and VC in CKD. Elevated sclerostin levels in serum are primarily derived from calcified smooth muscle cells. Reduced activity of the 1α-hydroxylase enzyme leads to increased sclerostin production in bones, which inhibit BMP-2 and osteogenic changes in arterial walls, potentially mitigating arterial calcification.

health [53]. This ligand is produced by stromal cells, osteoblasts, and osteocytes and is crucial for the differentiation of monocyte-macrophage osteoclast precursors into multinucleated osteoclasts and the activation of mature osteoclasts [53]. OPG, which functions as a soluble receptor for RANKL, predominantly exists in a free form and does not bind to cell membranes. By acting as a decoy receptor for RANKL, OPG impedes the maturation and formation of osteoclasts, thereby inhibiting bone resorption [53, 54]. Inhibition of the RANKL signaling pathway also improved the bone phenotype of adenosine-induced CKD-MBD mice. CKD mice with *RANKL* gene knockout did not have high levels of bone resorbing cells, nor did they form cortical porosity, indicating that RANKL is an important factor in CKD bone pathology and a potential therapeutic target for protecting bones in CKD [55].

Although the role of the RANKL-RANK-OPG signaling pathway in bone formation has been extensively studied, its regulatory function in VC remains largely unexplored. Mice lacking the *OPG* gene tend to develop early onset osteoporosis and VC, indicating the crucial role of the OPG/RANK/RANKL signaling pathway in connecting the bone and blood vessels. Administering recombinant OPG or inducing OPG overexpression in OPG-deficient mice mitigated osteoporosis and reversed arterial calcification [56]. OPG is produced by VSMCs and ECs and can potentially slow the progression of VC by binding to RANKL receptors. A prevalent hypothesis is that OPG produced by VSMCs, and ECs enters the bloodstream and exerts effects on distant bones, such as reducing bone resorption and affecting bone turnover. The anti-VC activity of OPG appeared to increase with increasing calcium load [19, 57].

Alkaline Phosphatase

ALP is a highly conserved enzyme that catalyzes the hydrolysis of phosphate monoesters, showing optimal activity at an alkaline PH. There are four forms of ALP isoenzymes present in the human body: tissue nonspecific ALP (also known as liver/bone/kidney ALP), intestinal ALP, placental ALP, and germ cell ALP. In the serum, the bone and liver ALP subtypes are the most abundant, with a ratio of approximately 1:1, constituting over 90% of the total ALP activity [58].

B-ALP is primarily produced by osteoblasts. B-ALP can be divided into four isoforms (B/I, B1x, B1, and B2), all of which are expressed in the human bone tissue and VSMCs. Notably, B1x is detected only in the serum of patients with CKD, especially exhibiting the highest activity in those on dialysis. B-ALP plays a crucial role in tissue mineralization and is expressed in osteoblasts, chondrocytes, and other mineralizing cell types (such as calcified VSMCs). One of the primary functions of B-ALP is to hydrolyze the mineralization inhibitor PPi into two phosphate molecules. In addition to inactivating PPi, B-ALP can deactivate the calcification inhibitor OPN through dephosphorylation, thereby modulating its activity. B-ALP is present in vesicles shed by VSMCs and contributes to calcification by promoting hydroxyapatite crystal deposition in the ECM [59].

Bone Morphogenetic Protein

BMPs are multifunctional growth factors of the transforming growth factor-beta superfamily. They play critical roles in various developmental processes including heart development, neurogenesis, and osteogenesis. Among the BMP family members, BMP-2 was the first to be characterized and is one of the most studied BMPs. It is pivotal during embryonic development and a powerful inducer of mesenchymal stem cell differentiation into osteoblasts, making it a key participant in bone formation [60].

VSMCs may also express BMP-2, which, in a BMPR-2-dependent manner, can promote monocyte infiltration and inflammation in atherosclerotic lesions and induce angiogenesis and the proliferation and migration of ECs. The upregulation of BMP-2 expression in dedifferentiated human VSMCs and the downregulation of BMP-2 agonists further support the idea that BMP-2 might engage in paracrine signaling, thereby facilitating inflammatory responses. Under BMP-2 stimulation, aortic smooth muscle cells can simultaneously express osteogenic marker Msx2 and smooth muscle markers, indicating that the transdifferentiation of fibroblasts into the osteoblast lineage may contribute to the development of VC [11].

Osteopontin

OPN is a transformation-related phosphoprotein in the SIBLING family and is encoded by the *SPP1* gene [61]. It exhibited a dual phenotype. OPN is secrete OPN, which can inhibit hydroxyapatite formation and promote bone resorption. Moreover, OPN can induce mineralization at sites of calcium accumulation. The expression of

OPN in cardiovascular diseases depends on its status. Although OPN is minimally expressed under normal conditions, its expression can increase significantly under various pathological conditions [62, 63].

The role and mechanisms of OPN in ectopic calcification have primarily been investigated in VC, focusing on two key aspects [64]. First, OPN can bind hydroxyapatite. In vitro studies have indicated that OPN effectively impedes the formation and expansion of hydroxyapatite crystals. OPNs specific RGD sequence of OPN encompasses a calcium-binding region abundant in aspartic acid. With negatively charged glutamic and aspartic acid residues, serine/threonine kinase substrate sites, and other calcium-binding motifs, OPN can bind substantial quantities of Ca^{2+} (up to 50 mol of calcium per mole of OPN). The second aspect pertains to how OPN facilitates monocyte uptake, thereby hindering calcification. OPN is synthesized by matrix or inflammatory cells at sites of ectopic calcification, where it adheres to biological hydroxyapatite, initially impeding crystal growth in a physical manner. This interaction between OPN and hydroxyapatite also furnishes recognition sites for macrophages and multinucleated giant cells, leading to local accumulation and upregulation of carbonic anhydrase II [64].

In 2021, Mace et al. [20] proposed a hypothesis that might explain the "calcification paradox." Using a rat model of 5/6 nephrectomy-induced VC, OPN was found to be the third most highly expressed gene in the aorta. Additionally, the expression of OPN overlapped with areas of calcification, suggesting that it might play a protective role in the local environment by preventing the deposition of calcium-phosphate crystals. This observation suggests that vascular changes in CKD could lead to defects in bone mineralization, limiting the deposition of calcium and phosphate in bones, which in turn could accelerate crystal formation within blood vessel walls. This creates a vicious cycle of bone demineralization and VC.

Runt-Related Transcription Factor 2

RUNX2 is essential for the proliferation of osteoblast precursor cells and immature osteoblasts, as well as for the upregulation of bone matrix molecules [65]. It is regarded as a key regulatory factor in bone development and is the primary trigger for osteoblast differentiation [66]. RUNX2 is mainly expressed in chondrocytes, osteoblast-lineage cells, and thymic cells, where it is crucial for chondrocyte maturation and osteoblast differentiation. In immature osteoblasts, RUNX2 regulates bone matrix protein genes, such as Col1a1, Col1a2, Spp1, Ibsp, and bone γ-carboxyglutamic protein (Bglap)/Bglap2, and induces osteoblast maturation [67]. Additionally, in vitro cell culture studies confirmed the key role of RUNX2 in vascular cell calcification, including its effects on VSMCs, ECs, and vascular progenitor cells. Specifically, elevated RUNX2 expression alone is sufficient to promote osteogenic differentiation and calcification in VSMCs. Although the specific mechanism by which RUNX2 promotes calcification in VSMCs is not entirely clear, insights from bone biology can help elucidate the pathways leading to RUNX2 upregulation in the vascular system. Major signaling pathways, such as BMP-2, ERK/MAPK, and PI3K/AKT, may contribute to VC and atherosclerosis by promoting the expression, post-translational modification, and transcriptional activity of RUNX2 [68].RUNX2 can also function as a part of the deoxyribo nucleic acid (DNA) damage response, linking DNA damage signals with osteogenic gene transcription and apoptosis, thereby promoting calcification. It localizes to DNA damage sites and is involved in DNA repair by regulating histone H_2AX phosphorylation [69].

CPPs and Calcification Propensity

CPPs act as a natural buffering system that helps prevent hypercalcemia and hyperphosphatemia by sequestering these ions. When mineral homeostasis is disturbed, elevated levels of calcium and phosphate can lead to the formation of circulating CPPs, which aggregate excessive ions into less harmful complexes [70]. There are two types of CPPs: primary and secondary CPPs. Primary CPPs (CPP1) are amorphous and soluble, and they are the predominant form of circulating CPP. In vitro, CPP1 spontaneously transforms into secondary CPPs (CPP2), which are larger and more crystalline. CPP2 may mediate the effect of phosphate on arterial calcification or directly induce oxidative stress in VSMCs leading to mineralization [71]. Larger CPP2 was associated with risk of mortality [71]. In summary, CPPs are essential for maintaining the balance of calcium and phosphate [72].

The primary-to-secondary CPP transformation time, also known as the serum T50, reflects the serum's endogenous ability to prevent calcium phosphate precipitation [73]. Higher T50 values indicate longer transition times and therefore a lower propensity for calcification [74]. T50 holds significant clinical relevance due to its

association with increased cardiovascular and all-cause mortality, as well as the progression of coronary artery calcification [75]. Additionally, T50 reflects the presence of other serum factors that contribute to the calcification process [76]. T50 is a critical factor in several CKD cohorts and has a potential to serve as a standard tool for evaluating VC [77]. A lower T50 or faster transformation of CPP1 to CPP2 has been demonstrated to be correlated with higher mortality rates [71].

Secondary CPPs exhibit the capacity to induce calcification in cultured vascular smooth muscle cells and innate immune responses in cultured macrophages, whereas primary CPPs lack such pathogenic activity. Specifically, secondary CPPs containing crystalline CaPi may serve as a pathogen for VC and chronic noninfectious inflammation in CKD patients [71, 78]. CPPs may exert influence on calcifying microvesicles-mediated calcification through several mechanisms. First, CPPs can induce apoptosis of VSMC, leading to the formation of apoptotic bodies which serve as a nidus for calcification. Second, CPPs are capable of causing an increase in cytoplasmic Ca^{2+} levels, and elevated cytosolic Ca^{2+} levels in VSMC contribute to the development of procalcifying calcifying microvesicles. Third, CPPs have been found to be present in calcified atherogenic lesions, where they may fuse with and integrate into the emerging microcalcifications [70].

CPPs cause Ecs dysfunction by impairing nitric oxide metabolism and generating oxidative stress [79]. The upregulation of H^+ and Ca^{2+} translocation, Ca^{2+} stress, generation of reactive oxygen species and oxidative stress, unfolded protein response, mitochondrial outer membrane permeabilization, and intrinsic apoptosis pathways in mitochondria and ER proteomes provide a framework for organelle-specific response after the internalization of CPP-1 or CPP-2 by the ECs [80].

T50 was moderately associated with mineral and inflammatory parameters but did not conclusively establish an association with BMD in HD patients [81]. It is demonstrated that blood CPPs possess the capability to exit from blood vessels in the bone and directly reach osteoblasts. It is not phosphate or calcium themselves, but rather CPPs that may be responsible for inducing fibroblast growth factor-23 secretion and production in osteoblasts or osteocytes [82].

The concept of a "bone-vascular axis" has been proposed, suggesting a complex interaction between the mechanisms that regulate both systems. It is suggested that fetuin-A and CPP may act as mineral chaperones in this interplay. The pathways involved in the production and accumulation of CPP are thought to be connected to the processes of bone function and remodeling, and may contribute to the paradoxical relationship between low BMD and increased VC, particularly in patients with CKD [83].

Treatment Prospect

The bone-vascular axis plays a central role in the pathogenesis of CKD-MBD. Researchers are actively exploring drugs or therapeutic methods that can antagonize bone metabolism-related proteins, with the aim of alleviating VC while addressing osteoporosis. Romosozumab is a monoclonal antibody used for the treatment of osteoporosis. It promotes bone formation by inhibiting the action of sclerostin, but it can exacerbate VC and increase the risk of cardiovascular events [52]. Therefore, the therapeutic goal for CKD-MBD is not to antagonize the activity of sclerostin, but rather to reduce its production by decreasing the osteogenic transdifferentiation of vascular smooth muscle cells [10].

Denosumab is a monoclonal antibody used primarily in the treatment of osteoporosis and certain types of bone-related conditions, such as bone metastases from cancer. It works by inhibiting RANKL, a protein essential for the formation, function, and survival of osteoclasts, the cells responsible for bone resorption [84, 85]. It resulted in increased BMDs at the lumbar spine and total hip, as well as a slight increase in calcified areas in a time-dependent manner [86]. However, some studies suggest that denosumab therapy has no effect on VC [87]. It even can potentially inhibit the progression of coronary artery calcification and lead to a reduction in osseous calcification, particularly in severe cases of high bone turnover [88]. Restoring the balance of RANKL-OPG by regulating the production of endogenous OPG may present a therapeutic approach for future prevention of bone loss and alleviation of VC [88].

Lanthanum carbonate, sucroferric oxyhydroxide, and etelcalcetide can reduce the burden of CPP1 and CPP2 in the serum of dialysis patients [89–91]. Hiroshi Kurosu1 and Makoto Kuro-o have proposed identifying CPPs as effective therapeutic targets. A CPP adsorption column made with alendronate can delay the occurrence of cardiovascular events without significantly lowering serum phosphate levels. This approach is aimed at establishing it as a medical device to improve clinical outcomes in dialysis patients [92].

Basic research targeting the bone-vascular axis is ongoing, but each study tends to focus solely on either the bone or the blood vessels [93], rather than considering them as a whole. As a result, effective treatment methods have yet to be discovered.

Conclusion

CKD-MBD is a complex multifactorial syndrome with no effective treatment currently available. Some scholars have proposed that inhibiting the transdifferentiation of VSMCs into osteoblast-like cells is fundamental to reducing VC, which may serve as a treatment goal in patients with CKD-MBD. Most current articles on the bone-vascular axis focus on the skeleton as an organ, where bone metabolic proteins are transported to blood vessels through various paracrine pathways. However, how blood vessels, in turn, influence the skeleton to drive the cycle of disease is an intriguing proposition that warrants further investigation.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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Author Contributions

Yingjing Shen was in charge of writing and revising the article. She also would provide critical feedback and revisions during the review process, which contributed significantly to the final version of the manuscript. Chen Yu also provided critical feedback and revisions during the review process, contributing significantly to the final version of the manuscript. Both authors read and approved the final manuscript.

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