



Vascular and Cardiac Valve Calcification in Chronic Kidney Disease

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Abstract

Vascular calcification (VC) and cardiac valve calcification (CVC) are the important causes to increase the risk of cardiovascular events in terms of chronic kidney disease (CKD) patients. Once VC and CVC considered a passive form of dead or dying cells, it has now emerged as a pathology results from an active and highly regulated cellular process. Recently, mechanisms of VC have been further elucidated and many of the pathways involved could be amplified in CKD patients. In particular, FGF-23/Klotho axis, Wnt pathways, PI3K/Akt signaling, P38MAPK signaling pathway, and microRNAs have been shown to be impaired among patients with CKD and could play a role during vascular calcification. Furthermore, risks for CVC in CKD patients and molecular mechanisms related to it were verified by several researchers. The scope of the present review is to summarize the risk factors and pathophysiological mechanisms potentially involved in the link between CKD and the progression of VC and CVC.

Keywords: Chronic kidney disease; Vascular calcification; Cardiac valve calcification; Mechanisms

Abbreviations CKD: Chronic Kidney Disease; VC: Vascular Calcification; CVC: Cardiac Valve Calcification; VECs: Valve Endothelial Cells; VICs: Valvular Interstitial Cells; FGF-23: Fibroblast Growth Factor 23; VSMCs: Vascular Smooth Muscle Cells; PTH: Parathyroid Hormone; FZD: Frizzled; LRP 5/6: Low density-lipoprotein-Receptor-related Protein 5/6; BMP-2: Bone Morphogenetic Protein 2; ESRD: End-Stage Renal Disease; PI3K: Phosphatidylinositol 3-Kinase; IM: Inflammatory Mediators; TNF: Tumor Necrosis Factor; ALP: Alkaline Phosphatase; siRNA: Small Interfering RNAs; AGEs: Glycation End Products; miRs: MicroRNAs; IL-6: Interleukin-6; EndMT: Endothelial-to Mesenchymal Transition; MGP: Matrix Gla Protein; GLA: Glutamic Acid; OPG: Osteoprotegerin

Introduction

Progression of chronic kidney disease (CKD) is associated with a lot of serious complications, including cardiovascular events which are the main cause of death in CKD patients [1]. Vascular calcification (VC) and cardiac valve calcification (CVC) are major causes of cardiovascular events [2,3]. Compared with the non-CKD population, the risk of vascular calcification or CVC in CKD is much higher than that in non-CKD, uncommonly increasing the chances of sudden death [3,4].

VC in CKD patients has two different but overlapping arterial pathologies: atherosclerosis and arteriosclerosis. The characteristics of atherosclerosis are lipid-laden plaques which limited to the tunica intima of the arterial wall, leading to vascular inflammation, thickening, as well as calcification [5]. Arteriosclerosis, known as medial arterial calcification, is accompanied by vascular fibrosis, thickening and stiffening, frequently contributing to left ventricular hypertrophy [6]. Heart valve consists mainly of valve endothelial cells (VECs) and valvular interstitial cells (VICs). Calcification of the valves

is mainly due to endothelial dysfunction, leading to interstitial cells loss and differentiation [7].

In this manuscript, we review the regulation of vascular and cardiac valvular calcification. We highlight mechanistic insights into mechanisms of VC and CVC and afford risk factors of CVC, which may provide the foundation for novel therapeutic approaches to treat vascular and cardiac valvular calcification in CKD.

Vascular Calcification Signaling in CKD

FGF-23/klotho axis

Fibroblast growth factor 23 (FGF-23), a bone-derived hormone, is located at 12p13 in humans, containing 251 amino acids protein (molecular weight=30 kDa), and it was widely considered as an important role in vascular changes [8,9]. Klotho, a part of klotho/FGF-receptor complex, was first described by Kuro-o et al. and then it began to be a vital part in health and disease [8,10-12]. It encodes a single-pass trans membrane klotho protein involved in cardiovascular disease, such as atherosclerosis and VC and expresses at high levels in renal distal tubular epithelium, and to a lesser extent in the parathyroid gland and human vascular tissue [12,13]. The membrane klotho interacts with fibroblast growth factor receptors (specially FGFR1) to form a high-affinity for FGF-23 to maintain the mineral homeostasis by inducing phosphate excretion into the urine and reducing the level of serum 1,25 (OH)₂D₃ [14,15]. However, the expression of klotho gene in kidney is located in the distal tubule, renal phosphate reabsorption mainly occurs in the proximal tubule. Thus, how FGF-23/klotho axis decrease phosphate resorption in the proximal needs to be further studied.

It has been demonstrated that high level of FGF-23 in vascular smooth muscle cells (VSMCs) and CKD which was related to the progression of artery calcification score independent of serum phosphorus level [16,17]. FGF23 is also associated with endothelial damage to the arteries especially in CKD [18,19]. Further studies

showed that active vitamin D and its analog against VC can be mediated by decreased FGF-23 and increased klotho expression independent of serum parathyroid hormone (PTH) level [20,21]. CKD is a state of vascular klotho deficiency promoted by chronic circulating stress factors, including pro inflammatory, uremic, and disordered metabolic condition, which can potentiate the development of human artery calcification and mediates resistance to FGF-23 [22,23]. Some people suggest that soluble klotho ameliorates VC by enhancing phosphaturia, preserving glomerular filtration and directly inhibiting phosphate uptake by vascular smooth muscle [23]. However, Cha et al. demonstrated that secreted klotho protein activates transient receptor potential vanilloid-5, responsible for calcium reabsorption in kidney, which can induce vascular calcification [24]. Thus, the relationship of klotho and vascular calcification remain unclear.

Canonical and non-canonical Wnt pathways

The Wnt pathways are a group of signal transductional pathways, which consist of the canonical Wnt pathway and the non-canonical Wnt/calcium pathway [25]. Canonical Wnt signaling pathway is activated when Wnt ligands (i.e. Wnt1, Wnt3a) bind to its receptors cell-surface Frizzled (FZD) and low density-lipoprotein-receptor-related protein 5/6 (LRP 5/6) [26,27]. The activation of FZD/LRP 5/6 receptor complex leads to the inactivation of GSK-3 β , and then β -catenin accumulate in the cytoplasm and trans-locate to the nucleus where β -catenin can heterodimerize with members of the lymphoid enhancer factor/T-cell factor family of transcription factors to induce the expression of specific genes [26-28].

Accumulating evidence has demonstrated that Wnt signal pathways are involved in vascular lesions, including endothelial dysfunction and migration, trans differentiation of VSMCs, and VC [29,30]. Wnt signaling is involved in high-phosphate and bone morphogenetic protein 2 (BMP-2) induced VSMC calcification [31,32]. We have demonstrated that increased expressions of β -catenin, GSK-3 β and Wnt-5a were observed in the calcific area of VC in end-stage renal disease (ESRD) patients and the logistic regression analysis indicated that Wnt-5a was an independent risk factor for vascular calcification in patients with ESRD [31]. Furthermore, PI3K/Akt has the ability to activate β -catenin signaling pathway by cross-linking MAPK signaling pathway to induce VC with CKD [33]. MAPK signaling pathway is a critical pathway which mediates eukaryote signal transmission and plays a crucial role in osteoblast differentiation and mineralization of VSMCs. Recent study reveals that P38MAPK can regulate canonical Wnt- β -catenin signaling pathway by inactivation of GSK-3 β in brain, thymus gland and spleen [34]. However, whether this pathway can be involved in calcification needs to be further studied.

PI3K/Akt signaling

Phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway is involved in inflammation, hyperphosphatemia and oxidative stress induced VC with CKD [35-37]. Okazaki et al. reveals that PI3K/Akt plays an inhibitory role in the inflammatory mediators (IM) including interferon-gamma, tumor necrosis factor (TNF)-alpha induced human VSMC calcification [38]. IM-induced alkaline phosphatase (ALP) activity in human VSMC can be attenuated or enhanced by wild-type or dominant-negative Akt respectively, and suppression of Akt with small interfering RNAs (siRNA) significantly reinforce ALP expression [38]. Hyperphosphatemia is a major risk factor for VC and cardiovascular mortality in CKD patients. Inorganic phosphate has been demonstrated to induce apoptosis and osteoblastic differentiation

of VSMCs, resulting to the development of VC through inhibiting gas6/Axl/PI3K/Akt pathway [39]. Patients with CKD are exposed to enhanced oxidative stress as a result of increased pro-oxidant activity and decreased anti-oxidant activity. This oxidative burden augments gradually with the development of CKD and plays a crucial role in the progress of apoptosis and osteoblastic differentiation of VSMC with CKD [40]. Byon et al. first discovered that exogenous H₂O₂ induced calcification of VSMCs through modulation of Runx2 by PI3K/Akt signaling, and inhibition of PI3K/Akt signaling blocked VSMC calcification and Runx2 expression concurrently [41,42]. Furthermore, xanthine oxidase induces VSMC calcification through PI3K/Akt signaling pathway [43].

P38MAPK signaling pathway

MAPK signaling pathway is a significant transduction pathway which participate in various cell physiological and pathology process including growth and differentiation. It mainly consists of four pathways covering ERK, JNK, P38MAPK, and ERK5/BMK1 [44]. Among those pathways, P38MAPK is considered to be closely related to VC with CKD. P38MAPK is mainly involved in hyperphosphate and oxidative stress induced VSMC calcification with CKD. CKD rats treated with Ca/P/VitD developed medial calcification of thoracic aorta where reactive oxygen species (ROS)-sensitive P38MAPK signaling was activated [45]. Inhibition of P38MAPK by inhibitors or siRNAs reduced Ca level and ALP activity in human SMCs treated with high Pi. Oxidized low density lipoprotein and advanced glycation end products (AGEs) are two kinds of oxidative stress products, which increased in the serum of CKD patients. Results from in vitro studies demonstrated that they can mediate VC via P38MAPK signaling pathway and the effect of AGEs can be suppressed by P38MAPK inhibitor [34,46].

MicroRNA

MicroRNAs (miRs) are small noncoding RNAs which regulate target gene expression via mRNA degradation, translational repression or mRNA alteration to influence cellular functions including proliferation, differentiation, and apoptosis [47-50]. Several studies have identified that miRs are associated with VSMC calcification. MiR-125b was down regulated in calcified aortas from apoE knockout mice, and its mimics can inhibit calcification of rat aortic SMCs cultured in high-phosphate medium [51,52]. MiR-30b and miR-30c were shown to be down regulated by BMP-2 in vitro, and the expression of miR-30b was also down regulated in calcified human coronary arteries [53]. Low miR-29 a/b expression was shown on calcific aortas from mice as well as on CKD patients [54].

Levels of miRNA-135a, miRNA-762, miRNA-714, and miRNA-712 were found to be higher in klotho mutant mice of VC than wild-type control, and their high levels were confirmed in VSMCs treated with calcium and inorganic phosphate [23,55]. The calcium efflux proteins NCX1, PMCA1, and NCKX 4 have been identified as potential targets of these miRs and inhibiting all four at the same time decreased calcium content by 30% by potentially reducing intracellular calcium loading [56]. The targets of miR-223 (Mef2c and RhoB) are known to play a role in VSMC contractility and differentiation, which is also involved in high phosphorus induced VSMC calcification [57]. MiR-221 and miR-222 were down regulated and act synergistically to induce calcification through cellular inorganic phosphate and pyrophosphate levels [58].

Accumulating evidence has confirmed that extracellular matrix vesicles contain non crystalline calcium and phosphate, as well as other factors related to VC, such as miRs [55,59]. RNA-seq analysis identified several miRs synthesized and packaged by porcine adipose tissue-derived mesenchymal stem cells, including miR148a, miR532-5p, miR378 and let-7f, enriched in matrix vesicles [60,61]. MiR-143/145 cluster can target myocardin or Kruppel-like factor-4 to mediate high phosphate-induced transition of SMCs to osteogenic cells [62,63]. It has also been reported to be involved in SMCs phenotype switch when SMCs were cultured with endothelial-derived vesicles [64]. In a study of 90 patients with CKD stage 3-4, circulating levels of miR-125b, miR-145 and miR-155, which target Runx2 and myocardin, decreased compared to those in healthy volunteers [65]. Other investigators found level of miR-15b decreased in 30 CKD patients and it was correlated positively with estimated glomerular filtration rate and negatively with phosphate levels [66]. Taken together, miRs in vesicles and its circulating form are also important for VC in CKD.

Risk Factors and Mechanisms of CVC in CKD

Dysregulation of phosphate and calcium

PTH, klotho, 1,25-(OH)₂D₃ and FGF-23 are basic regulators of calcium-phosphorus homeostasis. Most of the dietary phosphate can be absorbed by the gastrointestinal tract [67]. In the proximal tubules of kidney, PTH and FGF-23 increase phosphate excretion via the sodium-phosphate co-transporters. Meanwhile, klotho directly increases phosphaturia without FGF-23 [68]. 1,25-(OH)₂D₃ promotes intestinal phosphate absorption and also regulates PTH and FGF-23 [69]. Adeney et al. stated that serum phosphorus level is positive associated with VC by testing 439 CKD 3-4 stage patients and found that aortic valve calcification rate increased 33% while mitral valve reaching 62% when serum phosphorus increased 1 mg/dl [70]. When eGFR of CKD patients <60 ml/min, hyperphosphatemia happens, and high serum level of FGF-23 and PTH present in return to increase phosphate excretion. FGF-23 also reduces the activity of vitamin D by inhibiting 1 α hydroxylase directly [71]. In addition, increasing FGF-23 promotes left ventricular hypertrophy process, and then accelerates the deterioration of renal function in a vicious circle [72]. Besides, hyperphosphatemia can stimulate endothelial cells and then release endothelial microparticles, leading to inflammation and endothelial cell apoptosis [73]. Most CKD patients undergoing dialysis are accompanied with hypercalcemia, especially at the condition of application with calcium-containing phosphate binders. Patients with CKD always suffer from secondary hyperparathyroidism and high PTH increase intracellular calcium ion concentration, leading to mitochondrial oxidative stress and reducing ATP synthesis, which causes cell death, apoptosis and ectopic calcification [74].

Diabetes, hypertension and lipid metabolism disorders

Clinical studies have shown that vascular calcification rate is high for diabetics and the valve dysfunction for them presents a more serious condition [75]. The human cells including endothelial cells can be injured by the high blood glucose and carbohydrate metabolic products such as AGEs which can activate multiple signaling pathways (e.g. PI3K and JAK/STAT) and downstream factors (e.g. RANK) [76]. The hypertension incidence was about 70% among the investigated patients with CKD in China and the control of blood pressure was unsatisfactory [77]. The vasospasm contraction and endothelial dysfunction caused by hypertension can affect the synthesis and

secretion of the vessel dilators, and thus making the endothelial-dependent vasodilator response system worse [78]. The pathological studies of aortic valve diseases demonstrated that lipidosis and inflammatory infiltration are the most obvious pathological characteristics [79]. Therefore, hyperlipemia, hypertension and diabetes can cause the endothelial dysfunction and further promote valvular and vessel calcification.

Inflammation and oxidative stress

Inflammation and ROS are two common conditions associated with CVC in CKD patients. Inflammatory cytokines, such as the interleukin-6 (IL-6) superfamily and TNF superfamily, and inflammation-related transcription factor NF- κ B, have been reported to promote calcification in cultured VICs, VSMCs, or experimental animal models [80]. Leskinen et al. showed that IL-6 level is the risk factors for valvular calcification in CKD patients [81]. Furthermore, TNF release may trigger the Wnt signaling pathway, resulting CVC [82]. Miller et al. demonstrated that patients with calcification of aortic valve has high hydrogen peroxide content when compared with normal group, indicating that hydrogen peroxide mediated oxidative stress may play an important role in CVC [83]. Other researches have shown that hydrogen peroxide can directly stimulate nuclear binding factor1 (Cbfa1) and BMP2, thereby stimulating the differentiation of VICs into osteoblast-like cells [84].

Endothelial-to mesenchymal transition

In the early 1920s, Johannes Holtfreter defined the epithelial and endothelial-to mesenchymal transition (EMT/EndMT). It was found in the process of CVC [85]. In the early stage of valve calcification, the lesion accumulated with abundant sub endothelial lipids and extracellular matrix which can cause differentiation of VICs into osteoblast-like cells, one form of EndMT, as the disease worsens [86]. When EndMT occurs, calcium mucin mediated cellular interactions, reducing the EC differentiation. At the same time, the gene regulation of muscle fiber mother cell program is activated and differentiates into the osteoblast. BMP-Wnt- β -catenin pathway promotes EndMT by changing valve substrate environment, while VEC promote the occurrence of EndMT by increasing the expression of TGF- β family and activate the β -catenin pathway [87]. When EndMT happens, more VIC differentiates into osteoblast and the differentiation degree is directly related to calcification.

Fetuin-A

Fetuin-A is a calcium-binding glycoprotein present at high concentrations in human blood. Studies have shown that low level of serum fetuin-A is associated with aortic calcification, increasing cardiovascular mortality in patients with CKD [88]. Serum fetuin-A can inhibit mineral deposition by combining with minerals such as Ca²⁺, PO³⁺ forming particles which can be removed by reticulo endothelial circulation system, inducing CVC [89]. In addition, BMPs and TGF- β are suppressed by fetuin-A, reducing the effect on promoting calcification [90].

Matrix Gla protein

Matrix Gla protein (MGP) is an extracellular matrix protein isolated from the bone. It was the earliest calcification inhibitor which was discovered. The specific mechanism of MGP is still unclear in CKD. MGP can directly combine with hydroxylapatite and inhibit BMPs

[90]. MGP contains vitamin K dependent gamma carboxyl glutamic acid (GLA) residue, which has high affinity to the calcium ions and thus to prevent calcium deposition [91]. Because MGP is vitamin K dependent, clinical research demonstrated that patients with CKD and subclinical vitamin K deficiency have a high risk of CVC [92].

RANK/RANKL/OPG

There are three key elements that influence CVC: receptor activator of NF- κ B (RANK), receptor activator of NF- κ B ligand (RANKL), and osteoprotegerin (OPG). RANK, a type I membrane protein on the surface of osteoclast cells, is involved in osteoclast cell stimulation when bound with RANKL, and OPGL compete with RANKL inhibiting the activity [93]. In addition, lots of evidence suggests that the RANK/RANKL/OPG triad is involved in bone metabolism and may be important in CVC. Additionally, RANKL/RANK signal can also affect osteoclast activity. OPG protect bones by prevented the binding of RANKL and RANKL receptor ligand, resulting in inhibition of osteoclast differentiation, and preventing excessive bone reabsorption. Therefore, the ratio of RANKL/OPG is an important factor in CVC. RANK/RANKL promotes calcification while OPG inhibits calcification.

Wnt signaling

Wnt signaling pathway can adjust physiological bone formation. In CKD condition, pathological bone reabsorption triggers Wnt signaling, promoting osteogenesis and valvular calcification. Recent evidence suggests that Wnt signaling pathway related inhibitors which strengthen the function of osteoclasts and inhibits valvular calcification [94]. However, the function of Wnt signaling in bone formation and calcification is still poorly understood and underlying mechanism has not yet been well characterized.

Conclusions

Many patients with CKD have vascular or valvular calcification, which influence patients' survival rate seriously. Currently, there are very limited options for either the prevention or treatment of vascular or valvular calcification in CKD. Despite the fact that much insight has recently been gained into the mechanisms of ectopic calcification, further investigation and comprehension of this complicated process are still needed, especially for the interaction between VECs and VICs and their regulatory mechanism in the development of valve calcification. Only with better understanding of the pathophysiology of vascular and valvular calcification, will we find more effective therapeutic options for CKD patients.

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