Atherosclerosis: Open Access

Hao et al., Atheroscler open access 2016, 1:2

Review Article OMICS International

Vascular and Cardiac Valve Calcification in Chronic Kidney Disease

Lirong Hao*, Xueying Chang, Yuting Fu and Zhangxiu He

Department of Nephropathy and Hemodialysis, First Affiliated Hospital of Harbin Medical University, Harbin, China

***Corresponding author:** Lirong Hao, Department of Nephropathy and Hemodialysis, First Affiliated Hospital of Harbin Medical University, Harbin, China, E-mail: hao_lirong@163.com

Received date: September 19, 2016; **Accepted date:** November 01, 2016; **Published date:** November 08, 2016

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 Gowth 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 $(OH)_2D_3$ [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-3b, 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-3β 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-3β 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 ostoblastic 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 differenration of VSMC with CKD [40]. Byon et al. first discovered that exogenous H_2O_2 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-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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 1α 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 hypercalcinemia, 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-κB, 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 factorα1 (Cbfα1) 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 Ca^{2+}, PO^{3+} 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-kB (RANK), receptor activator of NF-kB 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.

References

1. London GM (2013) Mechanisms of arterial calcifications and consequences for cardiovascular function. Kidney Int Suppl 3: 442-445.
2. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, et al. (2003) Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 18: 1731-1740.
3. Wang AY, Ho SS, Wang M, Liu EK, Ho S, et al. (2005) Cardiac valvular calcification as a marker of atherosclerosis and arterial calcification in end-stage renal disease. Arch Intern Med 165: 327-332.
4. Hruska KA, Mathew S, Lund RJ, Menom I, Saab G, et al. (2009) The pathogenesis of vascular calcification in the chronic kidney disease mineral bone disorder: the links between bone and the vasculature. Semin Nephrol 29: 156-165.
5. Drüeke TB, Massy ZA (2010) Atherosclerosis in CKD: differences from the general population. Nat Rev Nephrol 6: 723-735.
6. Masho Y, Shigematsu T (2007) Arteriosclerosis and vascular calcification in chronic kidney disease (CKD) patients. Clin Calcium 17: 354-359.
7. Leopold JA (2012) Cellular mechanisms of aortic valve calcification. Circ Cardiovasc Interv 5: 605-614.
8. Olauson H, Vervloet MG, Cozzolino M, Massy ZA, Ureña Torres P, et al. (2014) New insights into the FGF23-Klotho axis. Semin Nephrol 34: 586-597.
9. Messa P (2014) FGF23 and vascular calcifications: another piece of the puzzle?. Nephrol Dial Transplant 29: 1447-1449.
10. Moe SM (2012) Klotho: a master regulator of cardiovascular disease?. Circulation 125: 2181-2183.
11. Kuro-o M (2012) Klotho in health and disease. Curr Opin Nephrol Hypertens 21: 362-368.
12. Kuro-o M (2010) Klotho. Pflugers Arch 459: 333-343.
13. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, et al. (2006) Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 444: 770-774.
14. Kitagawa M, Sugiyama H, Morinaga H, Inoue T, Takiue K, et al. (2013) A decreased level of serum soluble Klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease. PLoS One 8: e56695.
15. Van Ark J, Hammes HP, Van Dijk MC, Lexis CP, Van Der Horst IC, et al. (2013) Circulating alpha-klotho levels are not disturbed in patients with type 2 diabetes with and without macrovascular disease in the absence of nephropathy. Cardiovasc Diabetol 12: 116.
16. Zhu D, Mackenzie NC, Millan JL, Farquharson C, MacRae VE, et al. (2013) A protective role for FGF-23 in local defence against disrupted arterial wall integrity?. Mol Cell Endocrinol 372: 1-11.
17. Ozkok A, Kekik C, Karahan GE, Sakaci T, Ozel A, et al. (2013) FGF-23 associated with the progression of coronary artery calcification in hemodialysis patients. BMC Nephrol 14: 241.
18. Yilmaz G, Ustundag S, Temizoz O, Sut N, Demir M, et al. (2015) Fibroblast Growth Factor-23 and Carotid Artery Intima Media Thickness in Chronic Kidney Disease. Clin Lab 61: 1061-1070.
19. Rastogi A (2013) Sevelamer revisited: pleiotropic effects on endothelial and cardiovascular risk factors in chronic kidney disease and end-stage renal disease. Ther Adv Cardiovasc Dis 7: 322-342.
20. Lau WL, Leaf EM, Hu MC, Takeno MM, Kuro-o M, et al. (2012) Vitamin D receptor agonists increase klotho and osteopontin while decreasing aortic calcification in mice with chronic kidney disease fed a high phosphate diet. Kidney Int 82: 1261-1270.
21. Watson KE, Abrolat ML, Malone LL, Hoeg JM, Doherty T, et al. (1997) Active serum vitamin D levels are inversely correlated with coronary calcification. Circulation 96: 1755-1760.
22. Vervloet MG, Adema AY, Larsson TE, Massy ZA (2014) The role of klotho on vascular calcification and endothelial function in chronic kidney disease. Semin Nephrol 34: 578-585.
23. Lim K, Lu TS, Molostvov G, Lee C, Lam FT, et al. (2012) Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. Circulation 125: 2243-2255.
24. Cha SK, Ortega B, Kurosu H, Rosenblatt KP, Kuro-O M, et al. (2008) Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. Proc Natl Acad Sci U S A 105: 9805-9810.
25. Clevers H, Nusse R (2012) Wnt/α-catenin signaling and disease. Cell 149: 1192-1205.
26. He W, Dai C (2015) Key Fibrogenic Signaling. Curr Pathobiol Rep 3: 183-192.
27. Huang H, He X (2008) Wnt/beta-catenin signaling: new (and old) players and new insights. Curr Opin Cell Biol 20: 119-125.
28. Reis M, Liebner S (2013) Wnt signaling in the vasculature. Exp Cell Res 319: 1317-1323.

29. Cai T, Sun D, Duan Y (2016) WNT/beta-catenin signaling promotes VSMCs to osteogenic transdifferentiation and calcification through directly modulating Runx2 gene expression. Exp Cell Res 345: 206-217.
30. Wang Y, Li YP, Paulson C, Shao JZ, Zhang X, et al. (2014) Wnt and the Wnt signaling pathway in bone development and disease. Front Biosci (Landmark Ed) 19: 379-407.
31. Liu J, Zhang L, Zhou Y, Zhu D, Wang Q, et al. (2016) Aberrant activation of Wnt pathways in arteries associates with vascular calcification in chronic kidney disease. Int Urol Nephrol 48: 1313-1319.
32. Rong S, Zhao X, Jin X, Zhang Z, Chen L, et al. (2014) Vascular calcification in chronic kidney disease is induced by bone morphogenetic protein-2 via a mechanism involving the Wnt/beta-catenin pathway. Cell Physiol Biochem 34: 2049-2060.
33. Thornton TM, Pedraza-Alva G, Deng B, Wood CD, Aronshtam A, et al. (2008) Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation. Science 320: 667-670.
34. Tanikawa T, Okada Y, Tanikawa R, Tanaka Y (2009) Advanced glycation end products induce calcification of vascular smooth muscle cells through RAGE/p38 MAPK. J Vasc Res 46: 572-580.
35. Al-Aly Z, Shao JS, Lai CF, Huang E, Cai J, et al. (2007) Aortic Msx2-Wnt calcification cascade is regulated by TNF-alpha-dependent signals in diabetic Ldlr-/- mice. Arterioscler Thromb Vasc Biol 27: 2589-2596.
36. Shioi A, Katagi M, Okuno Y, Mori K, Jono S, et al. (2002) Induction of bone-type alkaline phosphatase in human vascular smooth muscle cells: roles of tumor necrosis factor-alpha and oncostatin M derived from macrophages. Circ Res 91: 9-16.
37. Tintut Y, Patel J, Parhami F, Demer LL (2000) Tumor necrosis factor-alpha promotes in vitro calcification of vascular cells via the cAMP pathway. Circulation 102: 2636-2642.
38. Okazaki H, Shioi A, Hirowatari K, Koyama H, Fukumoto S, et al. (2009) Phosphatidylinositol 3-kinase/Akt pathway regulates inflammatory mediators-induced calcification of human vascular smooth muscle cells. Osaka City Med J 55: 71-80.
39. Shioi A, Nishizawa Y (2009) Roles of hyperphosphatemia in vascular calcification. Clin Calcium 19: 180-185.
40. Nyitrai M, Balla G, Balla J (2015) Oxidative stress: one of the major causes of vascular calcification in chronic kidney disease patients. Orvosi hetilap 156: 1926-1931.
41. Byon CH, Javed A, Dai Q, Kappes JC, Clemens TL, et al. (2008) Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. J Biol Chem 283: 15319-15327.
42. Mody N, Parhami F, Sarafian TA, Demer LL (2001) Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. Free Radic Biol Med 31: 509-519.
43. Liu H, Li X, Qin F, Huang K (2014) Selenium suppresses oxidative-stress-enhanced vascular smooth muscle cell calcification by inhibiting the activation of the PI3K/AKT and ERK signaling pathways and endoplasmic reticulum stress. J Biol Inorg Chem 19: 375-388.
44. Blanc A, Pandey NR, Srivastava AK (2003) Synchronous activation of ERK 1/2, p38mapk and PKB/Akt signaling by H_2O_2 in vascular smooth muscle cells: potential involvement in vascular disease (review). Int J Mol Med 11: 229-234.
45. Agharazii M, St-Louis R, Gautier-Bastien A, Ung RV, Mokas S, et al. (2015) Inflammatory cytokines and reactive oxygen species as mediators of chronic kidney disease-related vascular calcification. Am J Hypertens 28: 746-755.
46. Liao L, Zhou Q, Song Y, Weikang Wu, Huimin Yu, et al. (2013) Ceramide mediates Ox-LDL-induced human vascular smooth muscle cell calcification via p38 mitogen-activated protein kinase signaling. PLoS One 8: e82379.
47. Huntzinger E, Izaurralde E (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 12: 99-110.
48. Djuranovic S, Nahvi A, Green R (2011) A parsimonious model for gene regulation by miRNAs. Science 331: 550-553.
49. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136: 215-233.
50. Rana TM (2007) Illuminating the silence: understanding the structure and function of small RNAs. Nat Rev Mol Cell Biol 8: 23-36.
51. Wen P, Cao H, Fang L, Ye H, Zhou Y, et al. (2014) miR-125b/Ets1 axis regulates transdifferentiation and calcification of vascular smooth muscle cells in a high-phosphate environment. Exp Cell Res 322: 302-312.
52. Goettsch C, Rauner M, Pacyna N, Hempel U, Bornstein SR, et al. (2011) miR-125b regulates calcification of vascular smooth muscle cells. Am J Pathol 179: 1594-1600.
53. Balderman JA, Lee HY, Mahoney CE, Handy DE, White K, et al. (2012) Bone morphogenetic protein-2 decreases microRNA-30b and microRNA-30c to promote vascular smooth muscle cell calcification. J Am Heart Assoc 1: e003905.
54. Du Y, Gao C, Liu Z, Wang L, Liu B, et al. (2012) Upregulation of a disintegrin and metalloproteinase with thrombospondin motifs-7 by miR-29 repression mediates vascular smooth muscle calcification. Arterioscler Thromb Vasc Biol 32: 2580-2588.
55. Johnson RC, Leopold JA, Loscalzo J (2006) Vascular calcification: pathobiological mechanisms and clinical implications. Circ Res 99: 1044-1059.
56. Gui T, Zhou G, Sun Y, Shimokado A, Itoh S, et al. (2012) MicroRNAs that target Ca(2+) transporters are involved in vascular smooth muscle cell calcification. Lab Invest 92: 1250-1259.
57. Rangrez AY, M'Baya-Moutoula E, Metzinger-Le Meuth V, Hénaut L, et al. (2012) Inorganic phosphate accelerates the migration of vascular smooth muscle cells: evidence for the involvement of miR-223. PLoS One 7: e47807.
58. Mackenzie NC, Staines KA, Zhu D, Genever P, Macrae VE, et al. (2014) miRNA-221 and miRNA-222 synergistically function to promote vascular calcification. Cell Biochem Funct 32: 209-216.
59. Davis-Dusenbery BN, Wu C, Hata A (2011) Micromanaging vascular smooth muscle cell differentiation and phenotypic modulation. Arterioscler Thromb Vasc Biol 31: 2370-2377.
60. Fiedler J, Stohr A, Gupta SK, Hartmann D, Holzmann A, et al. (2014) Functional microRNA library screening identifies the hypoxamir miR-24 as a potent regulator of smooth muscle cell proliferation and vascularization. Antioxid Redox Signal 21: 1167-1176.
61. Eirin A, Riester SM, Zhu XY, Tang H, Evans JM, et al. (2014) MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells. Gene 551: 55-64.
62. Davis-Dusenbery BN, Chan MC, Reno KE, Weisman AS, Layne MD, et al. (2011) Down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-beta and bone morphogenetic protein 4. J Biol Chem 286: 28097-28110.
63. Li Z, Hassan MQ, Volinia S, Van Wijnen AJ, Stein JL, et al. (2008) A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A 105: 13906-13911.
64. Hergenreider E, Heydt S, Treguer K, Boettger T, Horrevoets AJ, et al. (2012) Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol 14: 249-256.
65. Chen NX, Kiattisunthorn K, O'Neill KD, Chen X, Moorthi RN, et al. (2013) Decreased microRNA is involved in the vascular remodeling abnormalities in chronic kidney disease (CKD). PLoS One 8: e64558.
66. Wang H, Peng W, Ouyang X, Dai Y (2012) Reduced circulating miR-15b is correlated with phosphate metabolism in patients with end-stage renal disease on maintenance hemodialysis. Ren Fail 34: 685-690.
67. Marks J, Debnam ES, Unwin RJ (2010) Phosphate homeostasis and the renal-gastrointestinal axis. Am J Physiol Renal Physiol 299: F285-296.
68. Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, et al. (2010) Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. FASEB J 24: 3438-3450.

69. Sammels E, Parys JB, Missiaen L, De Smedt H, Bultynck G, et al. (2010) Intracellular Ca2+ storage in health and disease: a dynamic equilibrium. Cell Calcium 47: 297-314.

70. Adeney KL, Siscovick DS, Ix JH, Seliger SL, Shlipak MG, et al. (2009) Association of serum phosphate with vascular and valvular calcification in moderate CKD. J Am Soc Nephrol 20: 381-387.

71. Perwad F, Zhang MY, Tenenhouse HS, Portale AA (2007) Fibroblast growth factor 23 impairs phosphorus and vitamin D metabolism in vivo and suppresses 25-hydroxyvitamin D-1alpha-hydroxylase expression in vitro. Am J Physiol Renal Physiol 293: F1577-[1583].

72. Wesseling-Perry K, Jüppner H (2013) The osteocyte in CKD: new concepts regarding the role of FGF23 in mineral metabolism and systemic complications. Bone 54: 222-229.

73. Burger D, Levin A (2013) 'Shedding' light on mechanisms of hyperphosphatemic vascular dysfunction. Kidney Int 83: 187-189.

74. Pons F, Torregrosa JV, Fuster D (2003) Biological factors influencing parathyroid localization. Nucl Med Commun 24: 121-124.

75. Qasim AN, Rafeek H, Rasania SP, Churchill TW, Yang W, et al. (2013) Cardiovascular risk factors and mitral annular calcification in type 2 diabetes. Atherosclerosis 226: 419-424.

76. Wang Q, Song B, Jiang S, Liang C, Chen X, et al. (2015) Hydrogen Sulfide Prevents Advanced Glycation End-Products Induced Activation of the Epithelial Sodium Channel. Oxid Med Cell Longev 2015: 976848.

77. Xu H, Huang X, Risérus U, Cederholm T, Lindholm B, et al.(2014) Urinary albumin excretion, blood pressure changes and hypertension incidence in the community: effect modification by kidney function. Nephrol Dial Transplant 29: 1538-[1545].

78. Shimbo D, Muntner P, Mann D, Viera AJ, Homma S, et al. (2010) Endothelial dysfunction and the risk of hypertension: the multi-ethnic study of atherosclerosis. Hypertension 55: 1210-1216.

79. Butany J, Collins MJ, Demellawy DE, Nair V, Israel N, et al. (2005) Morphological and clinical findings in 247 surgically excised native aortic valves. Can J Cardiol 21: 747-755.

80. Hénaut L, Sanchez-Nino MD, Aldamiz-Echevarría Castillo G, Sanz AB, Ortiz A, et al. (2016) Targeting local vascular and systemic consequences of inflammation on vascular and cardiac valve calcification. Expert Opin Ther Targets 20: 89-105.

81. Leskinen Y, Paana T, Saha H, Groundstroem K, Lehtimäki T, et al. (2009) Valvular calcification and its relationship to atherosclerosis in chronic kidney disease. J Heart Valve Dis 18: 429-438.

82. Lacativa PG, Farias ML (2010) Osteoporosis and inflammation. Arq Bras Endocrinol Metabol 54: 123-132.

83. Miller JD, Chu Y, Brooks RM, Richenbacher WE, Peña-Silva R, et al. Dysregulation of antioxidant mechanisms contributes to increased oxidative stress in calcific aortic valvular stenosis in humans. J Am Coll Cardiol 52: 843-[850].

84. Byon CH, Javed A, Dai Q, Kappes JC, Clemens TL, et al. (2008) Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. J Biol Chem 283: 15319-15327.

85. Kovacic JC, Mercader N, Torres M, Boehm M, Fuster V, et al. (2012) Epithelial-to-mesenchymal and endothelial-to-mesenchymal transition: from cardiovascular development to disease. Circulation 125: 1795-1808.

86. Towler DA (2013) Molecular and cellular aspects of calcific aortic valve disease. Circ Res 113: 198-208.

87. Boström KI, Rajamannan NM, Towler DA (2011) The regulation of valvular and vascular sclerosis by osteogenic morphogens. Circ Res 109: 564-577.

88. Afsar CU, Uzun H, Yurdakul S, Muderrisoglu C, Ergüney M, et al. (2012) Association of serum fetuin-A levels with heart valve calcification and other biomarkers of inflammation among persons with acute coronary syndrome. Clin Invest Med 35: E206-[215].

89. Brylka L, Jahnen-Dechent W (2013) The role of fetuin-A in physiological and pathological mineralization. Calcif Tissue Int 93: 355-364.

90. Lomashvili KA, Wang X, Wallin R, O'Neill WC (2011) Matrix Gla protein metabolismin vascular smooth muscle and role in uremic vascular calcification. J Biol Chem 286: 28715-28722.

91. Leonard O, Spaak J, Goldsmith D (2013) Regression of vascular calcification in chronic kidney disease - feasible or fantasy? a review of the clinical evidence. Br J Clin Pharmacol 76: 560-572.

92. Shea MK, Holden RM (2012) Vitamin K status and vascular calcification: evidence from observational and clinical studies. Adv Nutr 3: 158-165.

93. Liu C, Walter TS, Huang P, Zhang S, Zhu X, et al. (2010) Structural and functional insights of RANKL-RANK interaction and signaling. J Immunol 184: 6910-6919.

94. Hampson G, Edwards S, Conroy S, Blake GM, Fogelman I, et al. (2013) The relationship between inhibitors of the Wnt signalling pathway (Dickkopf-1(DKK1) and sclerostin), bone mineral density, vascular calcification and arterial stiffness in post-menopausal women. Bone 56: 42-47.