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NEPHROLOGY - ORIGINAL PAPER

Aberrant activation of Wnt pathways in arteries associates with vascular calcification in chronic kidney disease

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Abstract
Purpose Development of vascular calcification in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) leads to increased cardiovascular morbidity and mortality. The mechanism of vascular calcification in CKD patients remains unclear. This study is aimed to evaluate the clinical association between abnormal Wnt pathways and incidence of vascular calcification in ESRD patients.
Methods A total of 41 ESRD patients were enrolled in this study. Tissue samples of radial arteries were obtained during arteriovenous fistula surgery. Expression of Wnt pathways was assessed by immunohistochemistry with antibodies against catenin, GSK-3beta and Wnt-5a. Correlation analysis was performed to evaluate the association between Wnt activities and vascular calcification rates.
Results Immunohistochemical stainings demonstrated that increased expressions of β-catenin, GSK-3beta and Wnt-5a were mostly observed in the subjects with vascular calcification. Further correlation analysis identified that β-catenin expression in overall arterial samples was significantly associated with the expressions of GSK-3beta and Wnt-5a. We also found significant correlation between expressions of GSK-3beta and Wnt-5a in the studied samples. The multivariate logistic regression analysis demonstrated that Wnt-5a was an independent risk factor for vascular calcification in patients with ESRD.
Conclusion Our study identifies increased activation of Wnt pathways in the arteries of patients with ESRD, which is significantly correlated with the incidence of vascular calcification. These findings support Wnt pathways as a potential target for future therapy of vascular calcification in CKD.

Keywords Chronic kidney disease · Wnt pathways · Vascular calcification · ESRD

Introduction

The worldwide rise in the incidence of chronic kidney disease (CKD) and consequent end-stage renal disease (ESRD) has reached epidemic proportions in the last decade [1]. CKD imposes substantial economic burden on affected individuals, especially in developing countries. Early detection of CKD can slow disease progression, reduce complications and risk of cardiovascular disease and improve survival and quality of life [2]. CKD is associated with high rates of cardiovascular mortality [3]. Cardiovascular disease is a major cause of morbidity and mortality in patients with CKD, especially in those with ESRD. The established cardiovascular risk factors do not account for the heightened risk associated with CKD. Novel risk factors, such as vascular calcification (VC), are known to be associated with increased mortality, presumably through development of LVH in CKD [4]. Moreover, VC has recently been identified as an independent risk factor for CKD progression and awaits definitive prospective outcome studies to fully validate its risk factor status [5, 6].

Early identification of VC in CKD is necessary to prevent disease progression and reduce the risk of cardiovascular morbidity and mortality. VC is one of the most

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common complications in patients with ESRD [7], which has been shown to be an independent predictor of vascular morbidity and mortality [8]. Emerging experimental and epidemiologic data indicate that vascular calcification likely begins much earlier, possibly as early as stage 2 of CKD [9, 10]. VC is defined as the inappropriate and pathological deposition of mineral in the form of calcium phosphate salts into the vascular tissues. VC associated with CKD is usually believed to be a passive process where the dysregulation of calcium and phosphorous resulted in deposition of mineral into the vessel wall [7]. VC remains prominent in patients with CKD, even with dietary phosphorus restriction and the therapy of well-tolerated, safe non-calcium-based binders [11]. A better understanding on the pathogenesis of VC is critical to develop better therapies for CKD patients at high risk of cardiovascular disease.

The Wnt pathways are a group of signal transduction pathways, which consist of the canonical Wnt pathway and the non-canonical Wnt/calcium pathway [12]. Emerging evidence suggests that Wnt pathways are involved in many aspects of the development and progression of vascular lesions, including endothelial dysfunction, macrophage activation and the migration of vascular smooth muscle cells (VSMCs) [13, 14]. In the animal models, Wnt pathways have been implicated in the pathogenesis of vascular calcification and osteoblastic transition in the VSMCs [15, 16]. Recent study reveals that Wnt/β-catenin pathways play a significant role in the process of high phosphorus-induced VSMCs calcification while knockdown expression of β-catenin results in reduced aortic calcification in the rat models [17]. Strikingly, reactivation of the Wnt pathways in early kidney disease produces elevations of circulating Wnt inhibitors, which cause the vascular dedifferentiation and vascular calcification in the CKD-MBD [9]. The accumulative data suggest a strong relationship between Wnt pathways and development of VC in patients with CKD. However, clinical evidence remains lacking to directly address whether activities of Wnt pathways correlate with VC development in CKD.

This study is aimed to evaluate the association between Wnt pathways and the presence of VC in patients with ESRD. As arterial tissues can provide the most reliable information on vascular biology, we suppose the expressions of β-catenin, GSK-3beta and Wnt-5a in radial arteries links to the development of VC in patients with ESRD, which might be a potential target for the treatment of vascular complication in CKD.

Materials and methods

Patients

This study was approved by the Institutional Review Board of the First Affiliated Hospital of Harbin Medical University. It was a retrospective study without any influence on the diagnosis and/or treatment. A total of 41 ESRD patients who underwent arteriovenous fistula plasty were enrolled in this study. Tissue samples of radial arteries (about 0.05–0.1 cm in diameter) were obtained from all subjects during the surgery. Tissue samples of femoral artery from trauma patients were used as negative controls for immunohistochemical analysis. All data were handled anonymously following guidelines of the institutional review board.

Immunohistochemical analysis

Immunohistochemistry was performed following the standard procedures of Pathology Department in the First Affiliated Hospital of Harbin Medical University. Briefly, all tissue samples were fixed with 10 % formaldehyde solution for 24 h after isolation and then proceeded with dehydration, impregnated, paraffin embedding and slicing with 3 μm thickness. Immunohistochemical stains were performed on formalin-fixed, paraffin-embedded sections with antibodies against GSK-3beta, β-catenin and Wnt-5a (antibodies ordered from *Wuhan Boster Biological Technology, Ltd., Wuhan, China*). Sections were rehydrated and antigens retrieved using heated citrate. Incubation of primary antibodies was performed same as previously described [18]. Staining was visualized using the labeled streptavidin biotin method. Related isotype immunoglobulins were used as negative controls in all stainings. All immunohistochemical analyses were repeated at least three times, and representative images were presented. An experienced pathologist from our center evaluated the degree of vascular calcification and grouped these samples based on the degree of calcium salt deposition.

Statistical analysis

Analysis was performed using SAS 9.3. Categorical variables were described as frequency (%). The Chi-square test was used to identify the different distributions of categorical variables between two groups. Cochran–Mantel–Haenszel (CMH) test was performed for ranked data with nonzero correlation analysis on the expression relevance among β-catenin, GSK-3beta and Wnt–5a. Continuous variables were presented as

Table 1 Baseline clinical characteristics of ESRD patients

Characteristic	Non-VC ($n = 32$)	VC ($n = 9$)	P
Gender			0.993
Male	19 (59.38)	6 (66.67)	
Female	13 (40.63)	3 (33.33)	
Hypertension, *n* (%)			0.103
No	11 (34.38)	0 (0.00)	
Yes	21 (65.63)	9 (100.0)	
Diabetes, *n* (%)			0.315
No	19 (59.38)	3 (33.33)	
Yes	13 (40.63)	6 (66.67)	
Age *n* (median, IQR)	50 (38, 60)	70 (51, 80)	0.010
Creatine (umol/l) (median, IQR)	694 (473, 895)	670 (642, 758)	0.625
Urea nitrogen (mg/dL) (median, IQR)	20.4 (13.6, 26.7)	25.2 (18.3, 32.4)	0.219
Ca2+ (mmol/L) (median, IQR)	2.04 (1.91, 2.25)	2.01 (1.77, 2.03)	0.157
Phosphate (mmol/L) (median, IQR)	1.69 (1.39, 2.12)	1.80 (1.35, 2.32)	1.000
iPTH (pg/ml) (median, IQR)	322 (121, 477)	369 (247, 480)	0.524
Cholesterol (mmol/L) (median, IQR)	4.2 (3.8, 5.0)	4.5 (3.7, 4.7)	0.956
Triglyceride (mmol/L) (median, IQR)	0.88 (0.74, 1.82)	1.31 (0.9, 1.71)	0.490
Albumin (g/L) (median, IQR)	35.3 (31.1, 40.2)	31.4 (28.8, 39.4)	0.798
Hemoglobin (g/L) (median, IQR)	89 (80, 107)	92, 84, 98)	0.910
PT(s) (median, IQR)	11 (10.2, 12.6)	10.5 (9.6, 10.8)	0.063
APTT(s) (median, IQR)	30 (26.3, 34.4)	32.4 (30, 33.2)	0.421
FIB(g/L) (median, IQR)	3.8 (3.1, 4.6)	4.6 (3.9, 5.21)	0.086
D-Dimer (μg/L) (median, IQR)	86 (1.09, 345)	363 (280, 450)	0.167

medians (interquartile range) and applied to Student's *t* test or Wilcoxon signed-rank test. Logistic regression analysis was used to measure the relationship between the dependent variable (vascular calcification) and independent variables such as age, GSK-3beta, β-catenin and Wnt-5a.

Results

Baseline clinical characteristics

As given in Table 1, the study population comprised 41 patients with established ESRD (male = 25, female = 16). By pathological evaluation, nine subjects were identified with establishment of VC in radial arteries, while 32 subjects with no detection of VC. No statistical difference in demographics was observed between the VC and non-VC groups, including gender ($P = 0.993$), incidence of hypertension ($P = 0.103$) and establishment of diabetes ($P = 0.315$). Intriguingly, serum levels of calcium ($P = 0.157$), phosphorous ($P = 1.000$) and intact parathyroid hormone ($P = 0.524$) were similar in both groups. The median age in VC group was higher than in non-VC group ($P = 0.010$).

Vascular expression of Wnt pathways members

Immunohistochemical stainings were performed to examine the activation of Wnt pathways. Representative staining images of Wnt-5a in radial arteries are shown in Fig. 1. We then analyzed the activities of Wnt pathways in radial arteries by immunohistochemical assays (Table 2). Expressions of β-catenin, GSK-3beta and Wnt-5a were analyzed in all VC patients. Enhanced expressions (++~+++) of β-catenin can be found in 78 % ($n = 7$) of subjects in VC group, but only 25 % ($n = 8$) in non-VC subjects

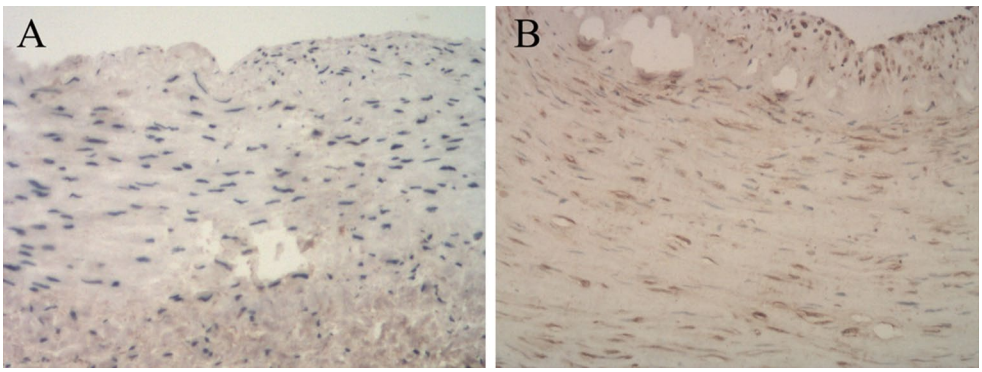

Fig. 1 Immunohistochemical staining of Wnt-5a in radial arteries of patients with ESRD. Representative images of Wnt-5a expression (*brown*) were shown in samples from **a** non-vascular calcification and **b** vascular calcification. Magnification ×100

Table 2 Expression assays of β-catenin, GSK-3beta and Wnt-5a by immunohistochemistry

Variables	Non-VC (%)	VC (%)	*P*
β-catenin			<0.001
+	24 (75.00)	2 (22.22)	
++	8 (25.00)	3 (33.33)	
+++	0 (0.00)	4 (44.44)	
GSK-3beta			<0.001
+	24 (75.00)	0 (0.00)	
++	8 (25.00)	4 (44.44)	
+++	0 (0.00)	5 (55.56)	
Wnt-5a			<0.001
+	28 (87.50)	2 (22.22)	
++	4 (12.50)	5 (55.56)	
+++	0 (0.00)	2 (22.22)	

($P < 0.001$). Similarly, increased expression of GSK-3beta (++~+++) was detected in all ($n = 9$) VC patients, while only 25 % of non-VCs ($n = 8$) were identified ($P < 0.001$). As a member of Wnt/Ca2+ signaling, enhanced expression of Wnt-5a was observed in 78 % ($n = 7$) of subjects in VC group, while 12.5 % of non-VCs ($n = 4$) were identified ($P < 0.001$). Significant differences in the expressions of β-catenin, GSK-3beta and Wnt-5a were detected between the two groups.

Correlation analysis on the Wnt pathways and VC

Given the importance of molecular interactions in different Wnt signalings [12], we next evaluated the relationships between examined markers. As a key factor in canonical Wnt pathways, β-catenin expression in overall enrolled subjects significantly correlated with those of GSK-3beta and Wnt-5a ($P < 0.001$) (Table 3). Furthermore, significant association was observed between the expressions of GSK-3beta and Wnt-5a ($P < 0.001$) (Table 4).

Lastly, we evaluated the value of Wnt pathways as an independent risk of VC establishment. All significant different factors identified between VC and non-VC subjects were further performed to multivariate logistic regression analysis (Table 5). Wnt-5a expression was significantly associated with the incidence of VC in ESRD patients ($P = 0.035$).

Discussion

In this study, we describe significant differences in the expressions of β-catenin, GSK-3beta and Wnt-5a between the VC and non-VC subjects (Fig. 1; Table 2), suggesting aberrant activation of Wnt pathways is involved in the development of VC in ESRD patients. Our study also highlights the importance of interplays between canonical and non-canonical Wnt pathways during the progression of VC in ESRD (Tables 3, 4). Moreover, we identify Wnt-5a as a potential risk factor for VC in ESRD (Table 5). Recent studies have revealed VC as an independent risk factor for cardiovascular mortality in CKD [19]. Its prevalence increases as renal function progressively deteriorates, especially in patients with ESRD [20]. Despite recent advances in our understandings of VC development, the exact nature of VC in CKD has yet to be clarified. Importantly, its clinical diagnosis is exclusively radiological since no sufficiently sensitive and specific biomarker of VC is

Table 3 Correlation analysis between β-catenin and GSK-3beta, Wnt-5a

Variables	β-catenin (%)			*P*
	+	++	+++	
GSK-3beta				<0.001
+	20 (76.92)	4 (36.36)	0 (0.00)	
++	6 (23.08)	5 (45.45)	1 (25.00)	
+++	0 (0.00)	2 (18.18)	3 (75.00)	
Wnt-5a				<0.001
+	23 (88.46)	7 (63.64)	0 (0.00)	
++	3 (11.54)	4 (36.36)	2 (50.00)	
+++	0 (0.00)	0 (0.00)	2 (50.00)	

Table 4 Correlation analysis between GSK-3beta and Wnt-5a

Variables	Wnt-5a			*P*
	+	++	+++	
GSK-3beta				<0.001
+	21 (70.00)	3 (33.33)	0 (0.00)	
++	9 (30.00)	2 (22.22)	1 (50.00)	
+++	0 (0.00)	4 (44.44)	1 (50.00)	

Table 5 Analysis of risk factors for VC

Variables	OR	95 % CI for OR		*P*
		Lower	Upper	
Age > 60 years versus ≤60 years	2.837	0.198	40.631	0.443
β-catenin "+++" and "++" versus "+"	1.001	0.050	19.902	0.999
GSK-3beta "+++" and "++" versus "+"	>999.999	<0.001	>999.99	0.938
Wnt-5a "+++"and "++" versus "+"	27.530	1.259	602.108	0.035

available at present [8, 21]. Several radiological methods, such as electron beam computed tomography, multislice spiral computed tomography and plain radiographs, have been used to investigate aortic calcification. Such methods are expensive, and none of them have been accepted as the gold standard in cardiovascular risk assessment. Our study shows that alteration of Wnt pathways in the arteries tissue may be related to VC in ESRD patients. In these patients, a number of Wnt members, including β-catenin, GSK-3beta and Wnt-5a, are markedly increased. Importantly, we used radial artery samples to evaluate the pathogenesis of VC in CKD, which can be more sensitive and specific to study the vascular injury than other serum origin biomarkers. Therefore, the identification of Wnt pathways markers in this study might offer a chance to make an early diagnosis and to assess the progression of VC, which would be a first step in the development of preventive and therapeutic tools to deal with the problem of VC in CKD patients.

VC is regulated in a manner similar to that of atherosclerotic calcification and promoted by the systemic inflammatory milieu [21, 22]. This pathophysiological process is also accompanied by VSMCs migration and endothelial activation that are triggered by multiple inflammatory pathways [23]. VC in CKD is associated with oxidative stress, uremia and hyperphosphatemia leading to the formation of osteoblast-like cells in the vessel walls. A breakthrough in this field is the recognition of its similarity to bone development and metabolism, in which endothelial, mesenchymal and VSMCs interact and respond to mechanical, inflammatory, metabolic and morphogenetic signals, which govern mineralization of connective tissues [24]. Thus, their counterparts in the artery wall may govern arterial mineralization. As a common complication in CKD, the extent of VC has been demonstrated as a predictor of subsequent vascular mortality. No longer can we accept the concept that VC in CKD is just a passive process resulting from an elevated calcium phosphate product. Rather, as a result of the metabolic insults of diabetes, dyslipidemia, oxidative stress, uremia and hyperphosphatemia, "osteoblast-like" cells form in the vessel wall [8, 25]. These mineralizing cells as well as the recruitment of undifferentiated progenitors to the osteochondrocyte lineage play a critical role in the calcification process. Thus, the simultaneous increase in arterial osteochondrocytic programs and reduction in active cellular defense mechanisms promote VC seen in ESRD. Innovative clinical therapy addressing this process requires understanding mechanisms in the context of a tightly controlled regulatory network, with multiple nested feedback loops and cross talk between organ systems.

The important role of the Wnt pathways in vascular disease has been demonstrated in recent studies that abnormal activities of Wnt components resulted in pathological conditions of vascular biology, such as atherosclerosis [26] and VC [27]. It has been demonstrated that endothelial BMP2 as well as BMP4 increases the expression of endothelial adhesion molecules in the endothelial cells, thus providing a potential link between BMPs activity and the stage of atherosclerotic lesion and VC formation [28]. Importantly, BMP2 regulates osteogenic and chondro-osteogenic transcription factors such as Msx homeobox 2 (Msx2) [29], which in turn upregulates the expression of multiple Wnt ligands and downregulates the endogenous inhibitor DKK1 [30]. As a result, the enhanced vascular expressions of canonical Wnt ligand, such as Wnt3a and Wnt7a, lead to canonical Wnt signal activation and VC. Of note, Wnt signaling is involved in the regulation of inflammation during atherosclerosis as well [31]. Multiple members of Wnt pathway, including β-catenin [32], GSK-3beta [33] and Wnt-5a [34, 35], have been reported to regulate a subset of inflammatory downstream genes in the progression of vascular inflammation. Novel roles for Wnt/β-catenin and Wnt/Ca2+ signaling in the regulation of endothelial inflammation have been suggested by aberrant Wnt-5a and DKK1 expression observed in human endothelial migration [36] and atherosclerotic plaques [27]. Kim et al. [37] recently described an interesting model for cooperative activation of endothelial and inflammatory cells that Wnt-5a induces inflammation in vascular endothelial cells by activating the NF-κB transcriptional pathway via the Ca2+-dependent non-canonical pathway. In line with these current findings, we observed significant activation of β-catenin, GSK-3beta and Wnt-5a in radial arteries with VC, but not in normal tissues, supporting that both Wnt/β-catenin and Wnt/Ca2+ signaling contribute to the development of VC in CKD.

At the same time, we do recognize that our study has several limitations. Firstly, this is a single-center study performed in a limited number of subjects of Han Chinese ethnicity. Emerging evidence indicates important racial differences in calcium metabolism in CKD patients, which might influence the vascular biology as well. Therefore, caution remains necessary to draw the same conclusion in other populations. Secondly, as an open-label study design, our results may be prone to observer bias, which could be minimized by use of clearly defined assessment standards in this study. Thirdly, we only examined the samples from patients with ESRD due to difficulties in obtaining appropriate tissues by invasive methods. The information of Wnt pathways in early CKD patients remains lacking, which might be addressed in the future with more advanced technique. Lastly, this study is not aimed to demonstrate the molecular events of Wnt pathways in CKD patients, which, however, remains technically challenging in the clinical study. Further well-designed animal studies with gene manipulations might help to reveal the mechanism of Wnt pathways in the development of VC.

Conclusion

In conclusion, to our best knowledge, this is the first clinical study specifically designed to investigate the correlation between vascular expressions of Wnt pathways and incidence of VC in patients with ESRD. Our data demonstrate increased activation of Wnt pathways in the radial arteries of patients with ESRD, including Wnt/β-catenin and Wnt/Ca2+ signaling. The expression patterns of Wnt pathways are significantly correlated with the incidence of VC in patients with ESRD. These findings highlight a key role of Wnt pathways in the pathogenesis of VC, which might be a potential target for future therapy of CKD.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Because it was a retrospective study without any influence on the diagnosis and/or treatment, no written consent was required.

References

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B et al (2013) Chronic kidney disease: global dimension and perspectives. Lancet 382:260–272
2. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJ, Mann JF et al (2013) Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. Lancet 382:339–352
3. Quarles LD (2013) Reducing cardiovascular mortality in chronic kidney disease: something borrowed, something new. J Clin Investig 123:542–543
4. Hruska K, Mathew S, Lund R, Fang Y, Sugatani T (2011) Cardiovascular risk factors in chronic kidney disease: does phosphate qualify? Kidney Int 79121:S9–S13
5. Torres PA, De Broe M (2012) Calcium-sensing receptor, calcimimetics, and cardiovascular calcifications in chronic kidney disease. Kidney Int 82:19–25
6. Drueke TB, Massy ZA (2016) Changing bone patterns with progression of chronic kidney disease. Kidney Int 89:289–302
7. Paloian NJ, Giachelli CM (2014) A current understanding of vascular calcification in CKD. Am J Physiol Ren Physiol 307:F891–F900
8. Mizobuchi M, Towler D, Slatopolsky E (2009) Vascular calcification: the killer of patients with chronic kidney disease. J Am Soc Nephrol JASN 20:1453–1464
9. Fang Y, Ginsberg C, Seifert M, Agapova O, Sugatani T, Register TC et al (2014) CKD-induced wingless/integration inhibitors and phosphorus cause the CKD-mineral and bone disorder. J Am Soc Nephrol JASN 25:1760–1773
10. Fang Y, Ginsberg C, Sugatani T, Monier-Faugere MC, Malluche H, Hruska KA (2014) Early chronic kidney disease-mineral bone disorder stimulates vascular calcification. Kidney Int 85:142–150
11. Sonikian M, Papachristou E, Goumenos DS (2013) Optimal use of phosphate binders in chronic kidney disease. Expert Opin Pharmacother 14:2521–2532
12. Clevers H, Nusse R (2012) Wnt/beta-catenin signaling and disease. Cell 149:1192–1205
13. Reis M, Liebner S (2013) Wnt signaling in the vasculature. Exp Cell Res 319:1317–1323
14. Wang Y, Li YP, Paulson C, Shao JZ, Zhang X, Wu M et al (2014) Wnt and the Wnt signaling pathway in bone development and disease. Front Biosci 19:379–407
15. Shao JS, Cheng SL, Pingsterhaus JM, Charlton-Kachigian N, Loewy AP, Towler DA (2005) Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. J Clin Investig 115:1210–1220
16. Sabbagh Y, Graciolli FG, O'Brien S, Tang W, dos Reis LM, Ryan S et al (2012) Repression of osteocyte Wnt/beta-catenin signaling is an early event in the progression of renal osteodystrophy. J Bone Miner Res 27:1757–1772
17. Yao L, Sun YT, Sun W, Xu TH, Ren C, Fan X et al (2015) High phosphorus level leads to aortic calcification via beta-catenin in chronic kidney disease. Am J Nephrol 41:28–36
18. Gross ML, Meyer HP, Ziebart H, Rieger P, Wenzel U, Amann K et al (2007) Calcification of coronary intima and media: immunohistochemistry, backscatter imaging, and x-ray analysis in renal and nonrenal patients. Clin J Am Soc Nephrol CJASN 2:121–134
19. Soriano S, Carmona A, Trivino F, Rodriguez M, Alvarez-Benito M, Martin-Malo A et al (2014) Endothelial damage and vascular calcification in patients with chronic kidney disease. Am J Physiol Ren Physiol 307:F1302–F1311
20. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY (2004) Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 351:1296–1305
21. Demer LL, Tintut Y (2008) Vascular calcification: pathobiology of a multifaceted disease. Circulation 117:2938–2948
22. Patsalas S, Eleftheriadis T, Spaia S, Theodoroglou H, Antoniadi G, Liakopoulos V et al (2007) Thirty-month follow-up of coronary artery calcification in hemodialysis patients: different roles for inflammation and abnormal calcium-phosphorous metabolism? Ren Fail 29:623–629
23. Antoniades C, Antonopoulos AS, Bendall JK, Channon KM (2009) Targeting redox signaling in the vascular wall: from basic science to clinical practice. Curr Pharm Des 15:329–342
24. Shanahan CM, Crouthamel MH, Kapustin A, Giachelli CM (2011) Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. Circ Res 109:697–711
25. Stevens KK, Patel RK, Mark PB, Delles C, Jardine AG (2015) Phosphate as a cardiovascular risk factor: effects on vascular and endothelial function. Lancet 385(Suppl 1):S10
26. Matthijs Blankesteijn W, Hermans KC (2015) Wnt signaling in atherosclerosis. Eur J Pharmacol 763:122–130
27. Marinou K, Christodoulides C, Antoniades C, Koutsilieris M (2012) Wnt signaling in cardiovascular physiology. Trends Endocrinol Metab TEM 23:628–636
28. Bostrom KI, Rajamannan NM, Towler DA (2011) The regulation of valvular and vascular sclerosis by osteogenic morphogens. Circ Res 109:564–577
29. Shao JS, Aly ZA, Lai CF, Cheng SL, Cai J, Huang E et al (2007) Vascular Bmp Msx2 Wnt signaling and oxidative stress in arterial calcification. Ann N Y Acad Sci 1117:40–50

30. Towler DA, Shao JS, Cheng SL, Pingsterhaus JM, Loewy AP (2006) Osteogenic regulation of vascular calcification. Ann N Y Acad Sci 1068:327–333
31. Tsaousi A, Mill C, George SJ (2011) The Wnt pathways in vascular disease: lessons from vascular development. Curr Opin Lipidol 22:350–357
32. DiRenzo DM, Chaudhary MA, Shi X, Franco SR, Zent J, Wang K et al (2016) A crosstalk between TGF-beta/Smad3 and Wnt/beta-catenin pathways promotes vascular smooth muscle cell proliferation. Cell Signal 28:498–505
33. Noh KT, Park YM, Cho SG, Choi EJ (2011) GSK-3beta-induced ASK1 stabilization is crucial in LPS-induced endotoxin shock. Exp Cell Res 317:1663–1668
34. Malgor R, Bhatt PM, Connolly BA, Jacoby DL, Feldmann KJ, Silver MJ et al (2014) Wnt5a, TLR2 and TLR4 are elevated in advanced human atherosclerotic lesions. Inflamm Res 63:277–285
35. Bhatt PM, Malgor R (2014) Wnt5a: a player in the pathogenesis of atherosclerosis and other inflammatory disorders. Atherosclerosis 237:155–162
36. Cheng CW, Yeh JC, Fan TP, Smith SK, Charnock-Jones DS (2008) Wnt5a-mediated non-canonical Wnt signalling regulates human endothelial cell proliferation and migration. Biochem Biophys Res Commun 365:285–290
37. Kim J, Kim J, Kim DW, Ha Y, Ihm MH, Kim H et al (2010) Wnt5a induces endothelial inflammation via beta-catenin-independent signaling. J Immunol 185:1274–1282