Secreted Frizzled-Related Protein 5 Ameliorates Vascular Calcification in a Rat Model of Chronic Kidney Disease through the Wnt/β-Catenin Pathway

Dai Deng  Xue Han  Zongli Diao  Wenhu Liu

Department of Nephrology, Beijing Friendship Hospital, Faculty of Kidney Diseases, Capital Medical University, Beijing, China

Keywords
Vascular calcification · Secreted frizzled-related protein 5 · Wnt/β-catenin pathway · Chronic kidney disease

Abstract
Introduction: Vascular calcification (VC) is highly prevalent and a major cardiovascular risk factor in chronic kidney disease (CKD) patients. Secreted frizzled-related protein 5 (SFRP5), an inhibitor of the Wnt pathway, is an adipokine with a positive effect on metabolic and cardiovascular diseases. Our previous in vitro study showed that SFRP5 attenuates high phosphate-induced calcification in vascular smooth muscle cells by inhibiting the Wnt/β-catenin pathway. Therefore, we hypothesized that SFRP5 may protect against CKD-associated VC (CKD-VC) through the same signalling. Methods: The rat model of CKD with VC was induced by 0.75% adenine combined with 1.8% high phosphate diet, which were administered with adenovirus vectors of SFRP5. We evaluated the SFRP5 effect on VC by von Kossa staining and calcium content analysis and osteogenic markers by immunohistochemistry and Western blot. The components of Wnt/β-catenin signalling were also evaluated. Results: SFRP5 local and serum levels were significantly decreased in the CKD-VC rat model compared with the control group. Adenovirus-mediated overexpression of SFRP5 significantly inhibited VC, which was due to suppression of CKD-induced expression of calcification and osteoblastic markers. Additionally, SFRP5 abrogated activation of the Wnt/β-catenin pathway that plays a major role in the pathogenesis of VC. Conclusion: Our results suggest that SFRP5 ameliorates VC of CKD rats by inhibiting the expression of calcification and osteoblastic markers as well as the Wnt/β-catenin pathway. Collectively, this study suggests that SFRP5 is a potential therapeutic target in CKD-VC.

Introduction

Chronic kidney disease (CKD) has become a public health problem worldwide and poses a serious threat to human health. CKD mineral and bone disorder is a common complication that contributes to the progression of vascular calcification (VC) [1, 2]. VC is an independent
risk factor that substantially contributes to cardiovascular events and increases the mortality of CKD patients [3, 4]. In addition to kidney damage, vascular stiffness caused by CKD-associated VC (CKD-VC) also leads to escalated cardiovascular mortality. In these patients, both intimal and medial calcification occurs, but medial arterial calcification is the most common. Although passive deposition of hydroxyapatites is considered the main pathological process of VC, recent studies have reported VC to be a complex process with involvement of various molecular pathways, which includes active transformation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells [4, 5]. However, the molecular mechanism of CKD-VC has not been fully elucidated.

Secreted frizzled-related protein 5 (SFRP5) is an anti-inflammatory secreted adipokine that belongs to the SFRP family [6]. SFRPs are inhibitors of the Wnt pathway, and SFRP5 inhibits either canonical or non-canonical signalling, which depends on the cell type. SFRP5 is a beneficial adipokine with protective effects on some cardiovascular diseases including VC [7–9]. Expression of SFRP5 in VSMCs and cardiomyocytes has also been demonstrated [10, 11]. Our previous in vitro study showed that SFRP5 interferes with the canonical Wnt pathway and mitigates high phosphate-induced VC [12]. A recent study found that serum SFRP5 levels were low in CKD patients with VC [13]. Therefore, SFRP5 might have a protective effect against CKD-VC. Because SFRP5 is an inhibitor of the Wnt/β-catenin pathway in VSMCs, we hypothesized that SFRP5 may protect against CKD-VC through the Wnt/β-catenin axis. To test our hypothesis, we established a CKD rat model to mimic clinical CKD-VC in vivo.

Materials and Methods

Animal Model

Eight-week-old male Sprague Dawley rats (250–300 g) were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (Beijing, China). Animals were housed in a climate-controlled room with a 12-h light/dark cycle and allowed free access to food and water. The animals were randomly assigned to 4 groups: (1) adenine− SFRP5−: normal diet + saline, n = 10; (2) adenine+ SFRP5−: adenine diet + saline, n = 10; (3) adenine+ Ad-SFRP5+: adenine diet + adenovirus-SFRP5, n = 10; (4) adenine+ Ad-GFP+: adenine diet + control adenovirus, n = 10. Standard chow (1.0% calcium [Ca] and 1.2% phosphorus [P]) was fed to control rats, and adenine-rich chow (0.75% adenine, 1.0% Ca, and 1.8% P; KEOAXIELI FEED Co., LTD., Beijing, China) was fed to rats for 6 weeks to induce CKD and VC. Then, rats were switched to a normal diet until 8 weeks. At the beginning of the experiment, the rats were administered the following treatments. Groups 1 and 2 were treated by tail vein injection of saline. Rats in group 3 received a tail vein injection of 2 × 10^11 plaque-forming units adenovirus-SFRP5 (National Human Genome Research Center, Beijing, China). Group 4 received a tail vein injection of 2 × 10^11 plaque-forming units control adenovirus. One week later, the injections were repeated. The rats were sacrificed at 8 weeks, and blood samples and aortic tissues were collected for various analyses. All animal procedures were carried out in accordance with local institutional and governmental regulations on the use of experimental animals.

Biochemical Parameters

Venous blood samples were collected in EDTA/acetic acid-containing tubes and centrifuged at 3,000 g for 10 min at 4°C. Serum levels of urea nitrogen (BUN), creatinine (Cr), Ca, P, and albumin were analysed using a Hitachi 7170 Autoanalyzer (Hitachi, Tokyo, Japan). The serum level of SFRP5 in rats was evaluated by using an SFRP5 micro-plate ELISA kit (Cloud Clone Corp, Wuhan, China) in accordance with the manufacturer’s instructions.

Quantification of Calcification

To quantitatively evaluate the degree of VC, frozen aortic tissue was weighed and hydrolysed in 1 mL of 6 mol/L hydrochloric acid for 24 h. The Ca content of the supernatant was determined using a commercially available kit (QuantiChromTM Calcium Assay Kit; Bioassay System, Hayward, CA, USA) and normalized to the wet tissue weight (µg/mg wet tissue weight). Additionally, aortic tissues were fixed with 4% paraformaldehyde, embedded in paraffin, cut into 3-µm-thick sections, and stained for calcification using a von Kossa staining kit (GenMed, Shanghai, China) in accordance with the manufacturer’s instructions. Dark brown staining indicated areas of calcification in vessel walls. Calcification was evaluated semiquantitatively by the calcification score (CS) using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The percentage of the calcified area was calculated as the von Kossa-positive area normalized to the total tissue area, which was measured in 10 representative areas and expressed as the mean value.

Immunohistochemistry

Paraffin-embedded sections of aortic tissues were incubated with primary antibodies against SFRP5 (1:100, ab230425; Abcam) and Runx2 (1:100, ab76956; Abcam) overnight at 4°C. Staining was performed using an ABC ELITE kit (Vector Laboratories, Burlingame, CA, USA) including biotinylated secondary antibodies in accordance with the manufacturer’s instructions. Sections stained
with secondary antibodies alone were confirmed to be negative. Image acquisition was performed using the analysis system described previously [14].

**Western Blot Analysis**
Preparation of thoracic aorta tissue homogenates and immunoblotting were performed as described previously [12, 14]. Primary antibodies and dilutions were as follows: anti-GAPDH (1:5000, ab181602; Abcam), anti-BMP-2 (1:1000, ab214821; Abcam), and anti-β-catenin (1:1000, ab6301; Abcam).

**Quantitative Real-Time PCR**
RNA isolation and quantitative real-time PCR (RT-PCR) were performed as described previously [14]. The primers used for PCR amplification are shown in Table 1. Quantitative RT-PCR was performed in duplicate with Power SYBR Green PCR Master Mix (Applied Biosystems) on an ABI 7500 sequence detection system in accordance with the manufacturer’s protocol. The mRNA levels of target genes were calculated after normalization to glyceraldehyde-3-phosphate dehydrogenase mRNA.

**Statistical Analysis**
Results are expressed as mean ± standard error of the mean. Differences between control and experimental groups were determined by one-way ANOVA and the Student-Newman-Keuls test for post hoc comparisons. All statistical analyses were performed using SPSS software (version 20.0). p < 0.05 was considered statistically significant.

**Results**

**Changes in Body Weight, Biochemical Analysis, and Serum SFRP5 Level**
During the study, untreated rats continued to gain weight, whereas rats that received the adenine diet consumed less food and lost body weight (Table 2). Animals fed adenine had dry hair and decreased activity. At the end of week 8, 1 rat had died in each adenine-fed group, except for the normal diet group.

As shown in Table 2, serum BUN and Cr concentrations at week 8 were increased significantly (p < 0.05), complicated by hypocalcaemia and hyperphosphataemia (p < 0.05) in adenine-fed rats compared with control rats. Additionally, an obvious decrease of SFRP5 was observed in adenine-fed rats (10.92 ± 3.56 vs. 21.33 ± 3.33 ng/mL, p < 0.01).

**Serum and Local Levels of SFRP5 in Rats by Different Treatments**
Because SFRP5 blocks VSMC calcification induced by high phosphate in vitro [12], we tested its therapeutic efficacy to mitigate VC in vivo. To investigate the relationship between SFRP5 and VC in adenine-fed CKD rats, we used an adenovirus to increase expression of SFRP5 as described in the Materials and Methods section. Adenovirus-mediated overexpression of SFRP5 increased the serum concentration of SFRP5 in CKD rats (18.22 ± 4.46 ng/mL), but there was no significant difference in the Ad-GFP group (10.85 ± 1.70 ng/mL) compared with the VC model group (10.92 ± 3.56 ng/mL) (Fig. 1a). The mRNA content of SFRP5 in white adipose tissue was higher than that in aortic tissue (4.51 ± 0.19 vs. 4.93 ± 0.81*), whereas aortic tissue (4.93 ± 0.81 vs. 4.96 ± 0.77*).

The expression of SFRP5 was localized in both endothelial and smooth muscle cells of vascular. The level in CKD rat aortas was lower than that in untreated rats, while Ad-SFRP5 could recover the SFRP5 expression as well (Fig. 1c). These results suggest that adenovirus-mediated overexpression of SFRP5 improves its level in both aortas and circulation.

**Table 2. Physiological and biochemical parameters of rats**

|                           | Adenine− Ad-SFRP5− (n = 10) | Adenine+ Ad-SFRP5− (n = 9) | Adenine+ Ad-SFRP5+ (n = 9) | Adenine+ Ad-GFP+ (n = 9) |
|---------------------------|-----------------------------|-----------------------------|-----------------------------|--------------------------|
|                           | baseline week 8              | baseline week 8              | baseline week 8              | baseline week 8          |
| Body weight, g            | 407.5±23.8                   | 491.9±33.1                  | 404.9±20.4                  | 405.8±19.7               |
| Food intake, g/kg/d       | 6.1±0.6                     | 5.8±0.6                     | 6.4±1.0                     | 5.5±0.4                  |
| Blood urea nitrogen, mmol/L | 4.68±0.56                   | 5.47±0.92                   | 5.23±0.93                   | 5.52±0.92                |
| Serum creatinine, μmol/L  | 35.3±1.6                    | 43.2±4.8                    | 36.1±2.5                    | 33.6±4.6                 |
| Serum calcium, mmol/L     | 2.50±0.09                   | 2.29±0.13                   | 2.50±0.04                   | 2.46±0.16                |
| Serum phosphate, mmol/L   | 3.22±0.13                   | 2.83±0.35                   | 3.21±0.16                   | 2.97±0.29                |
| Serum albumin, g/L        | 42.5±1.1                    | 39.0±2.7                    | 43.6±2.4                    | 44.2±2.2                 |

Data are mean ± SD. *p < 0.05 (vs. baseline).
Changes of VC in Rats by Different Treatments and the Protecting Effect of SFRP5

Figure 2 shows semiquantitative analysis of the aorta stained by the von Kossa method, which revealed the prevalence and degree of VC. In rats fed with adenine at week 8, VC was seen in the media layer of the aorta, whereas the calcification lesion was significantly attenuated after treatment with Ad-SFRP5. Staining was negative in untreated rats (Fig. 2a). To observe the degree of VC, the CS was calculated in accordance with the percentage of the calcified area in the vascular ring area (Fig. 2b; Table 3). The results also revealed that CS had declined in the Ad-SFRP5-treated group (4.26 ± 1.07 vs. 7.88 ± 0.74, p < 0.05) (Fig. 2c). Consistent with the von Kossa staining results, the aortic calcium content in Ad-SFRP5-treated rats at week 8 was lower than that in model and Ad-GFP-treated rats (4.01 ± 0.41 vs. 7.90 ± 0.31 and 7.21 ± 0.43, p < 0.05) (Fig. 2d).
Changes of Expression of Osteogenic Markers in the Aorta of Rats and the Inhibiting Effect of SFRP5

Figure 3 shows representative images of immunohistochemical staining and Western blot analysis of osteogenic markers Runx2 and BMP-2 in the aorta at week 8. In the group with intense calcification (model group), substantial expression of Runx2 and BMP-2 was observed in the aortic media. As expected, SFRP5 attenuated the induction of their expression (BMP-2, 1.81 ± 0.19 vs. 2.96 ± 0.25, p < 0.05, Fig. 3b).

Inhibition of the Wnt/β-Catenin Pathway by SFRP5 against VC

Our previous studies have shown that the protective effect of SFRP5 against calcification induced by high phosphate is mediated through inhibition of the canonical Wnt/β-catenin signalling pathway. In vivo experiments showed that SFRP5 suppressed β-catenin upregulation in calcification of the aorta in CKD rats (1.78 ± 0.33 vs. 3.19 ± 0.31, p < 0.05, Fig. 4a). Therefore, we determined whether SFRP5 also influenced downstream target genes of the Wnt/β-catenin signalling pathway in vivo. As
expected, quantitative RT-PCR revealed that increases in c-Myc and cyclin D1 expression in adenine-fed rats were reversed by SFRP5 (c-Myc, 1.08 ± 0.45 vs. 1.98 ± 0.23, \( p < 0.05 \), and cyclin D1, 1.32 ± 0.50 vs. 2.01 ± 0.24, \( p < 0.05 \), Fig. 4b).

**Discussion**

In this in vivo study, we established a rat model of VC in CKD induced by a diet with 0.75% adenine and 1.8% phosphorus, which is a common model of chronic renal failure. Previous studies have shown that serum BUN and Cr begin to increase after 2 weeks of adenine administration, and renal failure at 4 weeks cannot be reversed [15]. The animal model of chronic renal failure can be complicated with CKD mineral and bone disorder, that is, low calcium, high phosphorus, and high PTH. The middle layer of the aorta and coronary artery can be calcified at various degrees. Therefore, this model is widely used to study VC of CKD [16]. In this study, compared with the normal diet, serum BUN and Cr in the adenine diet group were increased significantly, and there was low calcium, high phosphorus, and aortic calcification. In the group complicated with VC, the aortic histomorphology and calcium content showed that calcification was significant and that the osteogenic trans-differentiation index was high in the VC model group.

Many recent studies have shown that Wnt signalling plays a major role in the progression of cardiovascular disease (CVD) [17], and SFRP5 acts primarily by inhibiting the Wnt signalling pathway, which suppresses the development of diseases [18]. Numerous studies have sup-

---

**Fig. 3.** SFRP5 inhibits the expression of osteogenic markers Runx2 and BMP-2 in the aorta of adenine-fed rats at week 8. **a** Representative photomicrographs (×400) of immunohistochemistry for Runx2. **b** Western blotting demonstrated that SFRP5 reversed the increased expression of BMP-2. Bars are the mean ± SEM of 9 samples per group. *\( p < 0.05 \) compared with the individual controls. SFRP5, secreted frizzled-related protein 5.

**Table 3.** Quantitative score of aortic calcification

| Calcification area/aortic ring area, S % | Calcification score |
|----------------------------------------|--------------------|
| 0 < S ≤ 10                             | 1                  |
| 10 < S ≤ 20                            | 2                  |
| 20 < S ≤ 30                            | 3                  |
| 30 < S ≤ 40                            | 4                  |
| 40 < S ≤ 50                            | 5                  |
| 50 < S ≤ 60                            | 6                  |
| 60 < S ≤ 70                            | 7                  |
| 70 < S ≤ 80                            | 8                  |
| 80 < S ≤ 90                            | 9                  |
| 90 < S ≤ 100                           | 10                 |
ported the protective effect of SFRP5 in the pathophysiology of CVD including VC [7–9]. VC is often detected in patients with CKD, which is highly correlated with its cardiovascular morbidity and mortality. Moreover, the serum concentration of SFRP5 is significantly lower in patients on haemodialysis with VC than in those without VC [13]. Our previous in vitro study showed that SFRP5 interferes with the canonical Wnt pathway and mitigates high phosphate-induced VC [12]. These findings suggest a suppressive effect of SFRP5 on CKD-VC. The effect of SFRP5 in vivo is relatively unexplored, but several recent studies have suggested that this may be an interesting area to investigate [11, 13]. Therefore, we investigated the role of SFRP5 through a series of in vivo experiments with a model of VC in adenine-induced CKD rats. The results showed that SFRP5 prevented CKD-VC and osteogenic differentiation, which was mediated by inhibition of the Wnt/β-catenin signalling pathway.

In the rat model of CKD-VC, we found that the serum concentration of SFRP5 was decreased significantly. Because Ouchi et al. [6] found that SFRP5 is highly expressed in adipose tissue, SFRP5 is considered to be secreted mainly from adipocytes. We measured the expression of SFRP5 in both white adipose and aortic tissues. The mRNA content of SFRP5 in adipose tissue was higher than that in aortic tissue, which is consistent with previous studies [6, 19]. Initially, SFRP5 was believed to be a secreted protein with autocrine or paracrine functions. Thus, we hypothesized that SFRP5 secreted primarily from adipocytes is released into systemic circulation. Serum SFRP5 levels are inversely correlated with inflammatory cytokine levels [20, 21], whereas decreased local production of SFRP5 in the CKD environment may also contribute to reduction of the serum SFRP5 level. Although the effect of SFRP5 on VC is relatively unexplored, our study suggests that SFRP5 expression changes within calcified vascular tissues, so that not only changes in serum levels of SFRP5, but also paracrine effects in or around vascular tissues are affected.

To investigate whether the decrease of SFRP5 was related to the occurrence of CKD-related VC, we used an adenovirus to overexpress SFRP5 and increase the level of SFRP5 in serum as well as adipose and aortic tissues. The results demonstrated that SFRP5 prevented CKD-VC. Von Kossa staining and calcium content analysis showed an increase in aortic calcification in CKD rats compared with controls. Moreover, SFRP5 treatment attenuated VC, and we confirmed the specificity of this ef-

**Fig. 4.** SFRP5 inhibited the activated Wnt/β-catenin pathway in CKD-associated VC. **a** Western blotting revealed that the expression of β-catenin significantly increased in the CKD with VC group compared with the control group, which could be inhibited by SFRP5 overexpression. **b** The mRNA levels of β-catenin transcriptional target genes c-Myc and cyclin D1 were consistent with the change of β-catenin. Bars are the mean ± SEM of 9 samples per group. *p < 0.05 compared with the individual controls. SFRP5, secreted frizzled-related protein 5; CKD, chronic kidney disease; VC, vascular calcification.
SFRP5 Ameliorates VC in CKD Rats

The inhibiting effects of SFRP5 are mostly involved in non-canonical Wnt signalling by antagonizing Wnt5a in metabolic syndrome-related diseases [27, 28]. The results of this study highlight a novel protective effect of SFRP5 against VC of CKD through inhibition of the canonical Wnt/β-catenin pathway. The cellular and signalling actions of SFRP5 appear to depend on the type of tissue as well as its inflammatory and metabolic states [29]. A previous study has suggested the Wnt β-catenin pathway promotes retinal vascularization and is accompanied by enhancement of β-catenin expression in the nucleus. A target gene of Wnt encodes angiogenic factor VEGF, which further validates the role of this pathway in the vascular system. Another study has reported that SFRP5 may inhibit VSMC proliferation, migration, and inflammation by suppressing Wnt/β-catenin [11]. SFRP5 also confers protection against oxidative stress-induced apoptosis through inhibition of β-catenin activation in human aortic endothelial cells [30].

Both plasma SFRP5 levels and SFRP5 expression in rat aortas increase with age via negative feedback and might play a protective role in vascular ageing. However, another recent study showed reduction of SFRP5 protein in the aorta of an animal model of adenine-induced CKD with VC, which was associated with activation of the non-canonical Wnt pathway [13]. Additionally, SFRP5 has an inhibitory effect on VSMC trans-differentiation into osteoblast-like cells, which is also involved in regulation of the Wnt3a-mediated non-canonical signalling pathway through inhibition of Rho/ROCK/JNK signalling. However, they also found β-catenin expression was also increased in CKD rats with VC. Unfortunately, this study did not further examine the effect of SFRP5 treatment on VC and the Wnt signalling pathway in animal models. This discrepancy may be partly due to the different methods to establish the model used in the 2 studies, among which high phosphorus is an important factor to induce VC in CKD. Additionally, the complex crosstalk between Wnt/β-catenin and non-canonical signalling pathways should be a focus. Therefore, further studies are required to better understand the crosstalk between these complicated signalling pathways in cells. Thus, in subsequent research, we will aim to determine how SFRP5 influences non-canonical Wnt signalling and conduct a more detailed analysis of the pathway components.

Conclusion

In summary, our results have shown that SFRP5 attenuates VC in an animal model of adenine-induced CKD and that this effect correlates with suppressed ex-
pression of calcification and osteoblast markers (BMP-2 and Runx2) through inhibition of the Wnt/β-catenin signalling pathway. The present study may facilitate elucidation of the role of SFRP5 in the pathophysiology of VC in CKD, which might be a therapeutic target for prevention or treatment of CKD-VC.

Acknowledgment

We thank Mitchell Arico from Liwen Bianji, Edanz Group, China (www.liwenbianji.cn/ac), for editing the English text of a draft of the manuscript.

Statement of Ethics

The experimental protocol was established according to the ethical guidelines of experimental animals and was approved by the Animal Ethics Committee of Beijing Friendship Hospital (No. YYYYDWSY191025).

References

1 Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group. KDIGO 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl. 2017;7:1–59.
2 Yamada S, Giachelli CM. Vascular calcification in CKD-MBD: roles for phosphate, FGF23, and Klotho. Bone. 2017;100:87–93.
3 Chen J, Budoff MJ, Reilly MP, Yang W, Rosas SE, Rahman M, et al. Coronary artery calcification and risk of cardiovascular disease and death among patients with chronic kidney disease. JAMA Cardiol. 2017;2(6):635–43.
4 Kakani E, Elyamny M, Ayach T, El-Husseini A. Pathogenesis and management of vascular calcification in CKD and dialysis patients. Semin Dial. 2019;32(6):553–61.
5 Hénaut L, Chillon JM, Kamel S, Massy ZA. Updates on the mechanisms and the care of cardiovascular calcification in chronic kidney disease. Semin Nephrol. 2018;38(3):233–50.
6 Ouchi N, Higuchi A, Ohashi K, Oshima Y, Gokce N, Shibata R, et al. Sfrp5 is an anti-inflammatory adipokine that modulates metabolic dysfunction in obesity. Science. 2010; 329(5990):454–7.
7 Cho YK, Kang YM, Lee SE, Lee Y, Seol SM, Lee WJ, et al. Effect of SFRP5 (Secreted Frizzled-Related Protein 5) on the WNT5A (Wingless-Type Family Member 5A)-induced endothelial dysfunction and its relevance with arterial stiffness in human subjects. Arterioscler Thromb Vasc Biol. 2018;38(6):1358–67.
8 Carstensen-Kirberg M, Kannenberg JM, Huth C, Meisinger C, Koenig W, Heier M, et al. Inverse associations between serum levels of secreted frizzled-related protein-5 (SFRP5) and multiple cardiometabolic risk factors: KORA F4 study. Cardiovasc Diabetol. 2017;16(1):109.
9 Miyoshi T, Doi M, Usui S, Iwamoto M, Kajiya M, Takeda K, et al. Low serum level of secreted frizzled-related protein 5, an anti-inflammatory adipokine, is associated with coronary artery disease. Atherosclerosis. 2014;233(2):454–9.
10 Jin X, Guo B, Yan J, Yang R, Chang L, Wang Y, et al. Angiotensin II increases secreted frizzled-related protein 5 (sFRP5) expression through AT1 receptor/Rho/ROCK1/JNK signaling in cardiomyocytes. Mol Cell Biochem. 2015;408(1–2):215–22.
11 Teliewubai J, Ji H, Lu Y, Bai B, Yu S, Chi C, et al. SFRP5 serves a beneficial role in arterial aging by inhibiting the proliferation, migration and inflammation of smooth muscle cells. Mol Med Rep. 2018;18(5):4682–90.
12 Deng D, Diao Z, Han X, Liu W. Secreted frizzled-related protein 5 attenuates high phosphate-induced calcification in vascular smooth muscle cells by inhibiting the Wnt/β-catenin pathway. Calcif Tissue Int. 2016;99(1):66–75.
13 Oh YJ, Kim H, Kim AJ, Ro H, Chang JH, Lee HH, et al. Reduction of secreted frizzled-related protein 5 drives vascular calcification through Wnt3a-mediated Rho/ROCK1/NK signaling in chronic kidney disease. Int J Mol Sci. 2020;21(10):3539.
14 Han X, Wang LY, Diao ZL, Liu WH. Apelin: a novel inhibitor of vascular calcification in chronic kidney disease. Atherosclerosis. 2016;244:1–8.
15 Katsumata K, Kusano K, Hirata M, Tsunemi K, Nagano N, Burke SK, et al. Sevelamer hydrochloride prevents ectopic calcification and renal osteodystrophy in chronic renal failure rats. Kidney Int. 2003;64(2):441–50.
16 Yamada S, Taniguchi M, Tokumoto M, Toyonaga J, Fujisaki S, Suehiro T, et al. The antioxidant tempol ameliorates arterial medial calcification in uremic rats: important role of oxidative stress in the pathogenesis of vascular calcification in chronic kidney disease. J Bone Miner Res. 2012;27(2):474–85.
17 Gay A, Towler DA. Wnt signaling in cardiovascular disease: opportunities and challenges. Curr Opin Lipidol. 2017;28(5):387–96.
18 Jaikanth C, Gurumurthi P, Indhumathi T, Cheriyan KM. Emergence of SFRP5 as a pleiotropic adipocytokine and its association with Wnt signaling pathways. Minerva Endocrinol. 2017;42(3):280–9.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study received financial support from Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding, Code: ZYLX201824.

Author Contributions

Dai Deng, Zongli Diao, and Wenhui Liu designed the experiment. Dr. Deng carried out and was a major contributor in manuscript writing. Xue Han performed the histological examination of the vascular. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.
19 Liu LB, Chen XD, Zhou XY, Zhu Q. The Wnt antagonist and secreted frizzled-related protein 5: implications on lipid metabolism, inflammation and type 2 diabetes mellitus. Biosci Rep. 2018;38(4):BSR20180011.
20 Almario RU, Karakas SE. Roles of circulating WNT-signaling proteins and WNT-inhibitors in human adiposity, insulin resistance, insulin secretion, and inflammation. Horm Metab Res. 2015;47(2):152–7.
21 Cheng L, Zhang D, Chen B. Declined plasma sfrp5 concentration in patients with type 2 diabetes and latent autoimmune diabetes in adults. Pak J Med Sci. 2015;31(3):602–5.
22 Rong S, Zhao X, Jin X, Zhang Z, Chen L, Zhu Y, et al. Vascular calcification in chronic kidney disease is induced by bone morphogenetic protein-2 via a mechanism involving the Wnt/β-catenin pathway. Cell Physiol Biochem. 2014;34(6):2049–60.
23 Li X, Yang HY, Giachelli CM. BMP-2 promotes phosphate uptake, phenotypic modulation, and calcification of human vascular smooth muscle cells. Atherosclerosis. 2008;199(2):271–7.
24 Montes de Oca A, Guerrero F, Martinez-Morenó JM, Madueño IA, Herencia C, Peralta A, et al. Magnesium inhibits Wnt/β-catenin activity and reverses the osteogenic transformation of vascular smooth muscle cells. PLoS One. 2014;9(2):e89525.
25 Yao L, Sun YT, Sun W, Xu TH, Ren C, Fan X, et al. High phosphorus level leads to aortic calcification via β-catenin in chronic kidney disease. Am J Nephrol. 2015;41(1):28–36.
26 Zhou P, Zhang X, Guo M, Guo R, Wang L, Zhang Z, et al. Ginsenoside Rb1 ameliorates CKD-associated vascular calcification by inhibiting the Wnt/β-catenin pathway. J Cell Mol Med. 2019;23(10):7088–98.
27 Nakamura K, Sano S, Fuster JJ, Kikuchi R, Shimizu I, Ohshima K, et al. Secreted frizzled-related protein 5 diminishes cardiac inflammation and protects the heart from ischemia/reperfusion injury. J Biol Chem. 2016;291(6):2566–75.
28 Chatani N, Kamada Y, Kizu T, Ogura S, Furuta K, Egawa M, et al. Secreted frizzled-related protein 3 (Sfrp3) decreases hepatic stellate cell activation and liver fibrosis. Liver Int. 2015;35(8):2017–26.
29 Tong S, Ji Q, Du Y, Zhu X, Zhu C, Zhou Y. Sfrp5/Wnt pathway: a protective regulatory system in atherosclerotic cardiovascular disease. J Interferon Cytokine Res. 2019;39(8):472–82.
30 Wang X, Peng Q, Jiang F, Xue L, Li J, Fan Z, et al. Secreted frizzled-related protein 5 protects against oxidative stress-induced apoptosis in human aortic endothelial cells via downregulation of Bax. J Biochem Mol Toxicol. 2017;31(12):e21978.