BMP7 Gene Silencing Inhibits Calcium Salt-Induced Osteogenic Differentiation of Porcine Aortic Valve Interstitial Cells Postprint

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Abstract

Objective: To investigate the effects and mechanisms of small interfering RNA (siRNA)-mediated silencing of bone morphogenetic protein 7 (BMP7) on calcium salt-induced osteogenic differentiation of porcine aortic valve interstitial cells, and to provide a theoretical basis for the intervention and treatment of calcific aortic valve disease (CAVD).

Methods: Non-CAVD valve tissues (non-CAVD group) were obtained from patients undergoing surgical treatment for aortic dissection, while CAVD valve tissues (CAVD group) were obtained from patients undergoing aortic valve replacement surgery for calcific aortic stenosis. Immunohistochemistry and Western blot were used to detect the protein expression levels of BMP7 and Runt-related transcription factor 2 (Runx2) in both groups. Aortic valve leaflets were immediately collected under sterile conditions from healthy domestic pigs after euthanasia, and aortic valve interstitial cells were isolated using sequential collagenase digestion. Morphological characteristics were observed, and phenotypic identification was performed using immunofluorescence staining. BMP7-siRNA was transfected into porcine aortic valve interstitial cells using liposome transfection, and changes in BMP7 expression were verified by qPCR and Western blot. After inducing osteogenic differentiation with calcium salt medium to establish an in vitro calcification model of aortic valve interstitial cells, ALP staining and alizarin red S staining were used to detect early and late osteogenic differentiation capabilities, respectively. qPCR and Western blot were used to detect the expression of osteogenesis-related genes and proteins Runx2, OCN, and OPN. Additionally, Western blot was used to detect the phosphorylation level of Smad1/5/8 in the BMP7 downstream signaling pathway.

Results: BMP7 and Runx2 protein expression levels were significantly higher in the CAVD group compared to the non-CAVD group. Primary porcine aor-

tic valve interstitial cells were successfully isolated, showing positive staining for -smooth muscle actin (-SMA) and vimentin, and negative staining for von Willebrand factor (vWF). After transfection with BMP7-siRNA, both mRNA and protein levels of BMP7 in porcine aortic valve interstitial cells were significantly downregulated, and both early and late osteogenic differentiation capabilities were significantly reduced. Silencing BMP7 gene expression downregulated the gene and protein expression of Runx2, OCN, and OPN, and significantly decreased the protein level of phosphorylated Smad1/5/8 (p-Smad1/5/8).

Conclusion: BMP7 gene silencing inhibits the osteogenic differentiation capability of calcium salt-induced aortic valve interstitial cells, and the BMP7/Smads signaling pathway may play an important role in this process.

Full Text

Preamble

BMP7 Gene Silencing Inhibits Osteogenic Differentiation of Porcine Aortic Valve Interstitial Cells Induced by Calcium Salts

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Abstract

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Methods: Non-CAVD valvular tissues (non-CAVD group) were obtained from patients undergoing surgical treatment for aortic dissection, while CAVD valvular tissues (CAVD group) were collected from patients receiving aortic valve replacement due to calcific aortic stenosis. Immunohistochemistry and Western blot were used to detect protein expression levels of BMP7 and Runt-related transcription factor 2 (Runx2) in both groups. Aortic valve leaflets were aseptically harvested from healthy domestic pigs immediately after sacrifice. Aortic valve interstitial cells were isolated using sequential collagenase digestion, and their morphological characteristics were observed followed by phenotypic identification via immunofluorescence staining. Porcine aortic valve interstitial cells were transfected with BMP7-siRNA using liposome-mediated transfection, and changes in BMP7 expression were verified by qPCR and Western blot. Cells were induced to undergo osteogenic differentiation using calcium salt culture medium

to establish an in vitro calcification model of aortic valve interstitial cells. Early and late osteogenic differentiation capacities were assessed by ALP staining and Alizarin red S staining, respectively. qPCR and Western blot were employed to examine expression of osteogenesis-related genes and proteins Runx2, OCN, and OPN. Additionally, Western blot was used to detect phosphorylation levels of Smad1/5/8 in the BMP7 downstream signaling pathway.

Results: BMP7 and Runx2 protein expression was significantly higher in the CAVD group compared to the non-CAVD group. Primary porcine aortic valve interstitial cells were successfully isolated, showing positive staining for -smooth muscle actin (-SMA) and vimentin, and negative staining for von Willebrand factor (vWF). Following transfection with BMP7-siRNA, both mRNA and protein levels of BMP7 in porcine aortic valve interstitial cells were markedly downregulated, accompanied by significantly reduced early and late osteogenic differentiation abilities. Silencing BMP7 expression downregulated both gene and protein expression of Runx2, OCN, and OPN, while protein levels of phosphorylated Smad1/5/8 (p-Smad1/5/8) were significantly decreased.

Conclusion: BMP7 gene silencing inhibits the osteogenic differentiation capacity of aortic valve interstitial cells induced by calcium salts, and the BMP7/Smads signaling pathway may play an important role in this process.

Keywords: calcific aortic valve disease; valve interstitial cells; BMP7; osteogenic differentiation; RNA interference

Introduction

Calcific aortic valve disease (CAVD) is a degenerative condition of the aortic valve characterized by fibroproliferative thickening and calcification of valve leaflets, leading to valve stiffening. In severe cases, it progresses to calcific aortic valve stenosis (CAVS), causing left ventricular outflow tract obstruction [1]. With population aging, CAVD has become the third most common cardiovascular disease in developed countries after coronary artery disease and hypertension, with a prevalence of 2.8% in individuals over 75 years that increases progressively with age [2]. An echocardiography-based random sampling survey of elderly populations in Beijing revealed that the incidence of cardiac valve calcification was 43.4% in those over 60 and as high as 82.3% in those over 70, predominantly affecting the aortic valve [3]. Currently, there are no effective pharmacological treatments for CAVD, and surgical aortic valve replacement remains the only effective therapy [4,5]. However, this approach carries high risks and costs associated with lifelong anticoagulation (mechanical valves) or reoperation (biological valves), and nearly one-third of patients cannot undergo surgery due to advanced age, poor health status, and multiple comorbidities [6,7]. Therefore, investigating the pathogenesis of CAVD is crucial for developing novel intervention and treatment strategies.

Valve interstitial cells (VICs) are fibroblast-like stem cells residing in heart valves that play essential roles in maintaining normal valve function [8]. Accumulat-

ing evidence demonstrates that VICs can differentiate not only into fibroblasts to replenish aging valvular fibroblasts but also into an osteogenic phenotype, making them the primary cell type involved in heart valve calcification [9].

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor- (TGF-) superfamily, named for their ability to induce bone and cartilage formation and ectopic bone formation in subcutaneous and muscular tissues. To date, 15 BMPs and 40 BMP-related proteins have been identified, which regulate proliferation, differentiation, and apoptosis of various cells, as well as embryonic development and organ formation. Histological studies of calcified aortic valves obtained during surgery have confirmed increased expression of BMP-2, BMP-4, and bone extracellular matrix molecules in calcified aortic valves [10], and in vitro studies by Osman et al. demonstrated that BMP2/4 significantly promotes expression of osteogenic markers—alkaline phosphatase (ALP), Runx2, and OPN —in VICs [11]. Therefore, accumulation of osteogenesis-related proteins is considered a primary pathogenic mechanism of valvular calcification leading to dysfunction, though the specific mechanisms remain unclear. Previous studies [12] have shown that metabolic syndrome and diabetes are associated with increased risk of aortic calcification, and numerous investigations have revealed important roles of BMP7 in age-related diseases such as renal hyperglycemic injury and prostate tumor progression [13-15]. The biological effects of BMP7 primarily involve binding to type II receptors, leading to phosphorylation of type I receptors, with signal transduction involving TGF- pathway transcription factors Smads, including receptor-regulated Smads (Smad1, Smad5), common Smad (Smad4), and inhibitory Smad (Smad6) [16]. However, no studies have reported on the role and mechanism of BMP7 in heart valve calcification.

Our study identified abnormally high BMP7 expression in calcified valvular tissue, leading us to hypothesize that BMP7 may be an important factor promoting valve calcification. Since osteogenic differentiation of valve interstitial cells is considered a critical step in heart valve calcification, we used porcine VICs as our primary research model. After inducing osteogenic differentiation with calcium salts in vitro, we interfered with BMP7 expression to investigate its effects on VIC osteogenic differentiation and underlying molecular mechanisms, providing new theoretical insights for CAVD intervention and treatment.

Materials and Methods

1.1 Materials

1.1.1 Sources of Human Valve Tissues and Porcine Valve Interstitial Cells Non-CAVD valvular tissues (non-CAVD group) were obtained from patients undergoing surgical treatment for aortic dissection at the First Affiliated Hospital of Chongqing Medical University, excluding those with fibrotic thickening or aortic regurgitation. CAVD valvular tissues (CAVD group) were collected from patients undergoing aortic valve replacement due to calcific aortic stenosis. After surgical removal, aortic valves were rinsed several times with

saline solution to observe valvular morphology. A portion of tissue was fixed in paraformaldehyde for morphological deformation score assessment, while the remainder was immediately stored in liquid nitrogen until sufficient samples were collected for experiments.

Porcine aortic valves were obtained from a designated animal quarantine slaughterhouse in Bishan District, Chongqing. Six healthy domestic pigs aged 8-10 months and weighing 100-120 kg were selected for aortic valve collection.

1.1.2 Major Reagents Type I collagenase, -glycerophosphate, streptomycin, penicillin, Alizarin red S dye, vitamin C, trypsin, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (USA). M199 medium was obtained from Hyclone (USA), and fetal bovine serum from Australia was purchased from Gibco. BMP7-targeting siRNA and negative control siRNA were obtained from Guangzhou RiboBio Co., Ltd. Rabbit anti-human -SMA, vWF antibodies, mouse anti-human vimentin, goat anti-rabbit IgG, and goat anti-mouse IgG were purchased from Wanlei Biotechnology. Mouse anti-human BMP7, p-Smad1/5/8, and Smad1/5/8 antibodies were from Santa Cruz Biotechnology. Rabbit anti-human Runx2, OCN, and OPN antibodies were from Abcam. Mouse anti-human GAPDH, horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit IgG were from Beijing Zhongshan Jinqiao Biotechnology. Polyvinylidene fluoride (PVDF) membranes and chemiluminescence kits were from Millipore. Trizol reagent, reverse transcription kits, and SYBR Green II were from TaKaRa. qPCR primers were synthesized by BGI Shenzhen. Alkaline phosphase (ALP) staining kit was from Beyotime Biotechnology. Other reagents were either imported or domestic analytical grade.

1.2 Methods

1.2.1 Isolation and Culture of Aortic Valve Interstitial Cells Within 30 minutes of sacrifice at the Bishan District animal quarantine slaughterhouse, aortic valve leaflets were aseptically harvested and immediately preserved in fresh medium containing double antibiotics. Samples were promptly transported to the laboratory, where valves were rinsed with PBS and incubated with 2 mg/mL Type I collagenase at 37°C for 30 minutes. Endothelial cells on the valvular surface were removed by sterile cotton swab scraping. Valves were then cut into 2 mm × 2 mm tissue fragments and incubated in collagenase overnight. The following day, cells were triturated, centrifuged, resuspended in M199 medium, and plated in culture dishes. Standard culture medium consisted of M199 supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were maintained at 37°C with 5% CO , with medium changed every other day. Cells were passaged upon reaching approximately 80% confluence. Well-characterized passages 3-6 of aortic valve interstitial cells were used for subsequent experiments.

1.2.2 Immunohistochemistry Tissue sections were deparaffinized and rehydrated. Antigen retrieval was performed by microwave treatment. Endogenous peroxidase activity was blocked with hydrogen peroxide, followed by serum blocking to reduce nonspecific background. Sections were incubated with primary antibody working solution overnight at 4°C, then with enzyme-conjugated secondary antibody for 30 minutes at room temperature. DAB solution was applied, and staining was monitored microscopically. When appropriate staining intensity was achieved, sections were rinsed in water to stop the reaction, counterstained with hematoxylin, dehydrated, cleared, and mounted.

1.2.3 Immunofluorescence Staining for Cell Phenotype Identification Primary aortic valve interstitial cells were cultured on coverslips until reaching 50% confluence. Cells were fixed with 4% paraformaldehyde for 30 minutes, permeabilized with 0.5% Triton X-100 for 20 minutes, and treated with 3% H O for 15 minutes to block endogenous peroxidase activity. After blocking with goat serum for 30 minutes at room temperature, cells were incubated with primary antibodies (rabbit anti-human -SMA, vWF, or mouse anti-human vimentin) diluted 1:100 overnight at 4°C. Fluorescent dye-conjugated secondary antibodies (goat anti-rabbit or goat anti-mouse IgG) diluted 1:100 were applied for 2 hours at room temperature in the dark. Finally, DAPI was used to counterstain nuclei, and expression of -SMA, vimentin, and vWF was observed under a fluorescence microscope.

1.2.4 Osteogenic Differentiation Induction of Valve Interstitial Cells Logarithmic-phase valve interstitial cells were cultured in calcium salt induction medium at 30-50% confluence for other treatments. The calcium salt induction medium was supplemented with 10 mM -sodium glycerophosphate and 50 g/mL vitamin C as conditioned medium to induce osteogenic differentiation.

1.2.5 siRNA Transfection and Experimental Groups Logarithmic-phase VICs were digested with 0.25% trypsin, centrifuged, resuspended, and seeded in 6-well plates at a density of 2 × 10 cells per well. When cells reached 70% confluence, they were transfected with interfering RNA, using non-transfected and negative control siRNA-transfected groups as blank and negative controls, respectively. After 24-48 hours of transfection, total cellular RNA was extracted for subsequent qPCR detection. After 48-72 hours, total cellular protein was extracted for subsequent Western blot detection. The negative siRNA template sequence was 5' -UUCUCCGAACGUGUCACGU-3' and antisense sequence was 5' -ACGUGACACGUUCGGAGAA-3' . The BMP7-siRNA template sequence was 5' -GGCUAUGCCGCCUACUACU-3' and antisense sequence was 5' -AGUAGUAGGCGGCAUAGCC-3' . Transfection experiments were divided into three groups: (1) Control group: VICs without any siRNA transfection; (2) Negative Control group: VICs transfected with negative siRNA; (3) BMP7-siRNA group: VICs transfected with BMP7-siRNA.

1.2.6 qPCR Detection of BMP7 and Osteogenic Differentiation Factor mRNA Expression Total RNA from each group was extracted using the TRIzol method and reverse-transcribed into cDNA using a two-step method for qPCR amplification. Using GAPDH as an internal reference, expression levels of BMP7, Runx2, OCN, and OPN were detected. The qPCR reaction system was 10 L with the following conditions: 95°C for 30 seconds; 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds; 72°C for 30 seconds. Experiments were repeated three times. Primer sequences are shown in Table 1 .

Table 1 Primer sequences (pig)

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Runx2	GACTTCAGCCTGGACAACGA	AGGTCCAGCATGAACATGGG
OCN	GCACTACCCAGCCACCTTTA	TATGGAGTGCTGCTGGTCTG
OPN	TCACACTGCTTGCCCTACTG	TGCCATAGAAGCGCCGATAG
BMP7	GAGCAAACAGACGATGTGGA	GACCAGCTCATCGGATTCAT
GAPDH	GGTGAAGGTCGGAGTGAACG	CGTGGGTGGAATCATACTGGA

1.2.7 Western Blot Detection of BMP7 and Osteogenic Differentiation Protein Expression Total protein was extracted from each group and protein concentration was determined by BCA assay. A protein loading amount of 250 g was used for each sample, and loading volume was calculated accordingly. Proteins were separated by SDS-PAGE and transferred to PVDF membranes at constant current (210 mA). Membranes were blocked with 5% BSA for 2 hours and incubated with primary antibodies overnight at 4°C (dilution ratios: BMP7 1:500, Runx2 1:500, OCN 1:1000, OPN 1:1000, p-Smad1/5/8 1:1000, Smad1/5/8 1:1000, GAPDH 1:1000). After washing, membranes were incubated with secondary antibodies for 1 hour at 37°C (dilution ratio 1:5000), washed again, and visualized by chemiluminescence. Gray values of each band were calculated using Quantity One software and normalized to GAPDH to determine protein expression levels. Experiments were repeated three times.

1.2.8 ALP Staining VICs were seeded in 24-well plates. When cell density reached 70%, different treatments were applied. After 6 hours, medium was replaced with working concentrations of 50 g/mL vitamin C and 10 mmol/L -glycerophosphate. After 7 days of culture, medium was removed and cells were rinsed twice with PBS, fixed with 100% ethanol at 4°C for 1 hour, and stained with 200 L NBT/BCIP solution per well. Staining results were observed after 30 minutes in the dark.

1.2.9 Alizarin Red S Staining VICs were seeded in 24-well plates. When cell confluence reached 70%, different treatments were applied. After 6 hours, medium was replaced with working concentrations of 50 g/mL vitamin C and 10 mmol/L -glycerophosphate, and cells were cultured for an additional 14 days

before Alizarin red S staining. Medium was removed, cells were rinsed three times with PBS, fixed with 0.05% glutaraldehyde at 4°C for 10 minutes, washed three times with deionized water, and stained with 0.4% Alizarin red S solution for 5 minutes. Stain was removed, and cells were washed with deionized water to stop the reaction before microscopic observation and imaging.

1.2.10 Statistical Analysis Experimental data were analyzed using SPSS 20.0 and GraphPad software. Measurement data were expressed as mean ± standard deviation (mean ± SD). Comparisons between two groups were performed using t-tests, while comparisons among multiple groups were performed using one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

2.1 BMP7 and Runx2 Protein Expression is Significantly Higher in Calcified Valvular Tissue than in Non-calcified Tissue

Immunohistochemistry results showed that BMP7 protein levels in the CAVD group were significantly higher than in the non-CAVD group Figure 1: see original paper. Western blot results demonstrated that BMP7 protein expression in the CAVD group was markedly elevated compared to the non-CAVD group ($P < 0.001$) Figure 1: see original paper. These results suggest that BMP7 may be a factor promoting valvular calcification.

Figure 1 BMP7 and Runx2 protein expression in the CAVD group was significantly higher than in the non-CAVD group. (a) Immunohistochemical detection of BMP7 and Runx2 expression in CAVD and non-CAVD groups (×200). Scale bar represents 50 m. (b) Western blot detection of BMP7 and Runx2 expression. Mean ± SD, n = 3. ***$P < 0.001$ vs. non-CAVD group.

2.2 Morphological Characteristics and Phenotypic Identification of Primary Porcine Aortic Valve Interstitial Cells

Cells completely adhered after 24 hours of seeding, exhibiting spindle or oval shapes with radial arrangement, and formed a confluent monolayer within approximately 5 days Figure 2: see original paper. Immunofluorescence staining revealed that the cultured cells expressed the interstitial cell-specific protein markers -SMA Figure 2: see original paper and vimentin Figure 2: see original paper in the cytoplasm, while showing no expression of the endothelial cell-specific marker vWF Figure 2: see original paper.

Figure 2 Morphological and phenotypic characterization of porcine aortic valve interstitial cells. (a) Light microscopy image of VICs (×100). (b) Immunofluorescence staining of -SMA (×200). (c) Immunofluorescence staining of vimentin (×200). (d) Immunofluorescence staining of vWF (×200).

2.3 Validation of BMP7-siRNA Transfection Efficiency in VICs

qPCR results showed that BMP7 mRNA expression in the BMP7-siRNA group was significantly reduced compared to both the blank control and negative control groups ($P < 0.01$) Figure 3: see original paper. Western blot results demonstrated that BMP7 protein expression in VICs of the BMP7-siRNA group was significantly decreased compared to the blank control and negative control groups ($P < 0.01$) Figure 3: see original paper. These results indicate that BMP7-siRNA transfection effectively interfered with BMP7 mRNA and protein expression in VICs.

Figure 3 Transfection with BMP7-siRNA decreased BMP7 expression in valve interstitial cells. (a) BMP7 mRNA expression detected by qPCR. (b) BMP7 protein expression determined by Western blot. Mean ± SD, n = 3. **$P < 0.01$ vs. control group or negative control group.

2.4 Downregulation of BMP7 Expression Inhibits Early and Late Osteogenic Differentiation Capacity of VICs

During osteogenic differentiation induction of VICs with osteogenic medium, ALP staining was significantly attenuated in the BMP7-siRNA group compared to the blank control and negative control groups at 7 days post-transfection Figure 4: see original paper. After 14 days of BMP7-siRNA transfection and osteogenic differentiation induction, Alizarin red S staining revealed markedly reduced calcium salt deposition in the BMP7-siRNA group compared to the blank control and negative control groups Figure 4: see original paper.

Figure 4 Effect of BMP7-siRNA on early and late osteogenic differentiation abilities of valve interstitial cells. (a) Early osteogenic differentiation capacity measured by ALP staining (×100). (b) Late osteogenic differentiation capacity measured by Alizarin red S staining (×100).

2.5 Downregulation of BMP7 Expression Inhibits Osteogenesis-Related Gene and Protein Expression in VICs

During osteogenic differentiation induction, qPCR results at 2 days post-transfection with BMP7-siRNA showed that expression levels of osteogenesis-related genes Runx2, OCN, and OPN in the BMP7-siRNA group were reduced compared to the blank control and negative control groups ($P < 0.01$, $P < 0.05$, $P < 0.05$, respectively) Figure 5: see original paper. Western blot results demonstrated that protein expression levels of Runx2, OCN, and OPN in VICs of the BMP7-siRNA group were significantly decreased compared to the blank control and negative control groups ($P < 0.01$, $P < 0.001$, $P < 0.01$, respectively) Figure 5: see original paper.

Figure 5 Downregulation of BMP7 inhibits expression of osteoblast-related genes and proteins in valve interstitial cells. (a) mRNA expression of Runx2, OCN, and OPN detected by qPCR. (b) Protein expression determined by West-

ern blot. Mean ± SD, n = 3. *$P < 0.05$,* ***$P < 0.01$,*** $P < 0.001$ vs. control group or negative control group.

2.6 Effect of BMP7 Downregulation on BMP-Smad1/5/8 Signaling Pathway

During osteogenic differentiation induction of VICs for 3 days, Western blot detection of protein expression showed that BMP7-siRNA significantly inhibited p-Smad1/5/8 protein expression levels compared to the control group ($P < 0.01$), while total Smad1/5/8 protein expression remained unchanged. These results suggest that BMP7 may regulate VIC osteogenic differentiation through the BMP-Smad1/5/8 signaling pathway.

Figure 6 Effect of BMP7-siRNA on BMP-Smad1/5/8 signaling pathway. Expression and activation of Smad1/5/8 protein detected by Western blot. $P < 0.01$ vs. control group or negative control group.

Discussion

With population aging, CAVD has become a major cardiovascular disease. Due to its insidious early onset, current detection methods are inadequate for early effective diagnosis. Once clinical symptoms appear, valve replacement surgery becomes the only option for patients. Therefore, investigating the pathophysiological mechanisms and exploring therapeutic targets for this disease are essential. Previously, heart valve calcification was considered a passive natural aging process. However, recent domestic and international reports have confirmed that heart valve calcification is an active process of ectopic calcification. Studies have demonstrated the presence of bone-related proteins in heart valves, suggesting that aortic valve calcification resembles osteogenesis—an active and potentially reversible pathological process [17] involving transdifferentiation of valve interstitial and endothelial cells, extracellular matrix remodeling, neovascularization, altered hemodynamic mechanical stress, lipid infiltration, calcium-phosphate metabolism disorders, and inflammatory stimulation [18,19].

As research on CAVD progresses, the role of the BMP protein family in its pathogenesis has received increasing attention. However, existing studies on BMPs in valve calcification have largely been limited to morphological staining observations without in-depth mechanistic elucidation of the roles played by BMPs during valvular calcification.

Our study found that BMP7 protein expression was significantly higher in calcified valvular tissue than in non-calcified tissue, leading us to hypothesize that BMP7 may promote VIC osteogenic differentiation and thereby contribute to CAVD development and progression. Due to limited availability of normal human aortic valve interstitial cells, we selected porcine aortic valve interstitial cells, which show high amino acid sequence homology with human BMP7 protein expression, as our research model for observing and investigating cell osteogenic

differentiation. During osteogenic differentiation induction of VICs, specific interference with BMP7 expression using siRNA significantly reduced both early and late osteogenic differentiation capacities, as demonstrated by ALP staining and Alizarin red S staining. qPCR and Western blot experiments revealed that interference with BMP7 markedly decreased expression of osteogenesis-related genes and proteins Runx2, OCN, and OPN. Concurrently, p-Smad1/5/8 levels were significantly reduced, suggesting that the canonical osteogenic signaling pathway BMP-Smads may be involved in BMP7-mediated regulation of VIC osteogenic differentiation. However, whether other osteogenic signaling pathways (such as Wnt- -catenin, Notch, MAPK, etc.) participate in this process remains unclear, and the specific mechanisms require further investigation.

In summary, this study demonstrates that BMP7 gene silencing significantly inhibits both early and late osteogenic differentiation capacities of VICs, and this mechanism may involve blockade of the BMP-Smads signaling pathway. Our findings provide a new potential therapeutic target for CAVD treatment. Inhibition of BMP7 expression in valve interstitial cells or blockade of the BMP-Smads signaling pathway may intervene in and delay the progression of valvular calcification. However, the specific mechanisms of BMP7 action require further investigation.

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Note: Figure translations are in progress. See original paper for figures.