Acta Biochim Biophys Sin 2022, 54(12): 1863–1873
https://doi.org/10.3724/abbs.2022179
Advance Access Publication Date: 6 December 2022
Original Article

Original Article

Deletion of large-conductance calcium-activated potassium channels promotes vascular remodelling through the CTRP7-mediated PI3K/Akt signaling pathway

Jing Bi[1,2,†], Yanru Duan[1,2,†], Meili Wang[1,2], Chunyu He[1,2], Xiaoyue Li[1,2], Xi Zhang[1,2], Yan Tao[1,2], Yunhui Du[3], and Huirong Liu[1,2,*]

[1]Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China, [2]Beijing Key Laboratory of Metabolic Disturbance Related Cardiovascular Disease, Beijing 100069, China, and [3]Beijing Key Laboratory of Upper Airway Dysfunction-Related Cardiovascular Diseases, Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, China

[†]These authors contributed equally to this work.

*Correspondence address. Tel: +86-10-83911830; E-mail: liuhr2000@126.com

Received 11 April 2022 Accepted 14 June 2022

Abstract

The large-conductance calcium-activated potassium (BK) channel is a critical regulator and potential therapeutic target of vascular tone and architecture, and abnormal expression or dysfunction of this channel is linked to many vascular diseases. Vascular remodelling is the early pathological basis of severe vascular diseases. Delaying the progression of vascular remodelling can reduce cardiovascular events, but the pathogenesis remains unclear. To clarify the role of BK channels in vascular remodelling, we use rats with BK channel α subunit knockout (BK $\alpha^{-/-}$). The results show that BK $\alpha^{-/-}$ rats have smaller inner and outer diameters, thickened aortic walls, increased fibrosis, and disordered elastic fibers of the aortas compared with WT rats. When the expression and function of BK α are inhibited in human umbilical arterial smooth muscle cells (HUASMCs), the expressions of matrix metalloproteinase 2 (MMP2), MMP9, and interleukin-6 are enhanced, while the expressions of smooth muscle cell contractile phenotype proteins are reduced. RNA sequencing, bioinformatics analysis and qPCR verification show that C1q/tumor necrosis factor-related protein 7 (*CTRP7*) is the downstream target gene. Furthermore, except for that of MMPs, a similar pattern of IL-6, smooth muscle cell contractile phenotype proteins expression trend is observed after *CTRP7* knockdown. Moreover, knockdown of both *BK α* and *CTRP7* in HUASMCs activates PI3K/Akt signaling. Additionally, CTRP7 is expressed in vascular smooth muscle cells (VSMCs), and BK α deficiency activates the PI3K/Akt pathway by reducing CTRP7 level. Therefore, we first show that BK channel deficiency leads to vascular remodelling. The BK channel and CTRP7 may serve as potential targets for the treatment of cardiovascular diseases.

Key words large-conductance calcium-activated potassium channel, vascular remodelling, vascular smooth muscle cell, C1q/tumor necrosis factor-related protein 7

Introduction

The large-conductance calcium-activated potassium (BK) channel is widely distributed in the cardiovascular system [1] and can participate in the maintenance of membrane potential and regulate vascular contraction/relaxation [2]. BK channels are considered to be key players in the vascular system. The abnormal expression/activity of BK channels is related to a variety of cardiovascular diseases, such as hypertension and premature uterine artery contraction [3]. However, the roles of the BK channel in cardiovascular systems are not well understood.

Vascular remodelling is one of the early detectable parameters that predicts life-threatening cardiovascular events. This pathological feature is found in many patients with hypertension and atherosclerosis and may be involved in the development and

complications of numerous vascular diseases, including myocardial ischemia, coronary heart disease, and heart failure [4,5]. In fact, a high degree of arterial remodelling predicts a poorer prognosis in patients with vascular diseases [6]. However, the pathophysiological mechanisms of vascular remodelling are complicated and have not yet been elucidated. It has been demonstrated that vascular inflammation, reduced expressions of smooth muscle cell (SMC) contractile phenotype proteins, changes in extracellular matrix components and vascular function are pathological features of vascular remodelling [7]. Our previous study demonstrated that the blood vessels of BK channel α subunit-knockout (BK $\alpha^{-/-}$) rats exhibited enhanced vasoconstriction [8]. Some other studies showed that BK channel deficiency stimulated an increase in the release of inflammatory factors in the blood and reduced the expressions of SMC contractile phenotype proteins [9,10], indicating that abnormal BK channel expression or function may be involved in vascular-related injury. Unexpectedly, although BK channels play a significant role in vascular structure and function, we have been unable to directly define the relationship between BK channels and vascular remodelling.

In the present study, we used BK $\alpha^{-/-}$ rats to show that BK channel deficiency is involved in vascular remodelling. Meanwhile, the potential molecular mechanism of vascular remodelling was preliminarily explored *in vitro*, and we found that C1q/tumor necrosis factor-related protein 7 (CTRP7) was expressed in smooth muscle. Loss of the BK channel α subunit reduced CTRP7 expression, thereby activating the PI3K/Akt signaling pathway. This study revealed the mechanism of vascular remodelling, and targeting BK channels and CTRP7 might represent potential strategies for vascular disease prevention and treatment.

Materials and Methods

Animals

The animals used in this study are BK channel α subunit (*KCNMA1*)-knockout and wild-type (WT) rats. In this study, the method of construction, reproductive mode and genetic identification of male BK $\alpha^{-/-}$ rats were the same as previously described [11]. The animals (24 weeks) were fed in the SPF animal room of Capital Medical University. At the end of the experiment, the rats were euthanized with an intraperitoneal injection of sodium pentobarbital. All animal experiment protocols used in this study were approved by the Animal Research Ethics Committee of Capital Medical University (Licence Number: AEEI-2018-049). The heart rate and blood pressure of rats were monitored with a BP-98 A tail-cuff blood pressure measurement system (Softron, Tokyo, Japan) when the animals were in a conscious state.

Cell culture

Human umbilical artery smooth muscle cells (HUASMCs) were purchased from Shanghai Xinyu Biotechnology Company (Shanghai, China). HUASMCs were maintained in Dulbecco's modified Eagle's medium (DMEM; 10-014-cv; Corning, New York, USA) containing 10% fetal bovine serum (FBS; FSS500; Excell Bio, Shanghai, China) at 37°C in an incubator with 95% air and 5% CO_2 after they reached 80% confluence. To examine the effect of BK channels on HUASMCs, the medium was supplemented with the 1, 5, and 10 μM BK channel inhibitor paxilline (ab141840; Abcam, Cambridge, UK). After 24–72 h of treatment, the cells were collected for subsequent studies.

Small interfering RNA (siRNA)-mediated gene knockdown

BK α siRNA, CTRP7 siRNA and scramble siRNA were purchased from RiboBio Company (Guangzhou, China). When SMCs reached 50% confluence, the cells were transfected with siRNA in the presence of Lipofectamine 2000 transfection reagent (11668019; Invitrogen, Carlsbad, USA) according to the manufacturer's protocol (final siRNA concentration: 50 nM). Successful knockdown was confirmed by western blot analysis of BK α and CTRP7 protein levels. Because BK α-1 siRNA and CTRP7-1 siRNA had high knockdown efficiency, they were selected for subsequent experiments. The siRNA sequences are shown in Table 1. The negative control (siNC; Cat. No. P201911180066) was purchase from Ribobio Company (Guangzhou, China)

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from SMCs using Trizol reagent (T9424; Sigma-Aldrich, St Louis, USA). cDNA was generated using a Revert Aid First Strand cDNA Synthesis kit (K1622; Thermo Scientific, Waltham, USA) according to the manufacturer's instructions. qRT-PCR was performed using a SYBR Green qPCR Master Mix kit (ES-QP002; ESsciense Biotech,Beijing, China), and the results were normalized to *β-actin* mRNA level. All amplification reactions were performed as follows: one cycle of 5 min at 95°C to activate the polymerase, followed by 40 cycles of 95°C for 10 min and 60°C for 30 s. Each sample was assayed three times. The primer sequences are shown in Table 2.

Western blot analysis

Cell or tissue lysates were resolved by 10% SDS-PAGE and then transferred onto nitrocellulose membranes. After being blocked with 5% milk in Tris buffered solution-Tween (TBST), the membranes were incubated with primary antibodies at 4°C overnight, followed by incubation with the corresponding secondary antibodies for 1 h. The intensities protein bands were analysed using the ChemiDoc MP Imaging System (Bio-Rad, Hercules, USA). The primary antibodies used are as follows: anti-Slo1/BKAppha potassium channel (1:500; 75-022-020; Neurolab, St Louis , USA), anti-GAPDH (1:1000; TA309157; ZSGB-BIO Beijing, China), anti-MMP9 (1:1000; ab76003; Abcam), anti-MMP2 (1:1000; ab92536; Abcam), anti-IL-6 (1:500; ab9324; Abcam), anti-Calponin1 (1:1000; ab46794; Abcam), anti-SM-22α (1:1000; ab14106; Abcam), anti-CTRP7 (1:1000; A00396-01-100; Aviscera Bioscience, Santa Clara, USA), anti-FAK (1:1000; T55464; Abmart, Shanghai, China), anti-p-FAK (phospho Y397) (1:1000; ab81298; Abcam), anti-AKT (1:500; 10176-2-AP; Proteintech, Chicago, USA), anti-p-AKT (phospho S473) (1:1000; ab81283; Abcam), anti-Smad2 (1:1000; 5339T; Cell Signaling Technology, Beverly, USA), and anti-p-Smad2 (1:1000; 3108T; Cell Signaling Technology). The secondary antibodies are HRP-conjugated goat anti-rabbit IgG (1:5000; ZB-2301) or anti-mouse IgG (ZB-2305).

Table 1. Sequence of siRNAs used in this study

siRNA	Species	Sequence (5′→3′)
BK α-1	*Homo sapiens*	GGCAGAAATACTACTTGGA
BK α-2	*Homo sapiens*	CATCGGTGCACTTGTAATA
CTRP7-1	*Homo sapiens*	GTGGAAGCATCGTGCTCAA
CTRP7-2	*Homo sapiens*	GGGCAATACCGGATAAAGA

Table 2. Sequences of primers used for real-time PCR

Gene	Species	Forward primer (5′→3′)	Reverse primer (5′→3′)
β-actin	*Rattus norvegicus*	GAGACCTTCAACACCCCAGCC	TCGGGGCATCGGAACCGCTCA
FZD2	*Rattus norvegicus*	TCGTTTTGCCCGTCTCT	TAGCGGAATCGCTGCAT
KBTBD8	*Rattus norvegicus*	GCCGCGTCGGCAGATTT	TCCCGTGATCCACTTCCACT
PLCE1	*Rattus norvegicus*	TGCATTCTTGCTCCAGTCTCT	TTGAGTGTTTTCTGTTCCCTGTA
CTRP7	*Rattus norvegicus*	CCAGAGCACGAGCCCAAG	AGTAGCTCTCCCCCTTAGCC
β-actin	*Homo sapiens*	CCACGAAACTACCTTCAACTCC	TCATACTCCTGCTGCTTGCTGATCC
MCP-1	*Homo sapiens*	CAAACTCAAGCTCGCACTC	CATTTCCACAATAATATTTTAG
MMP-9	*Homo sapiens*	TGTCCCTTTACTGCCCTGA	ACTCCAGGCTCTGTCCTCCTCTT
MMP-2	*Homo sapiens*	TGATCTTGACCAGAATACCATCGA	GGCTTGCGAGGGAAGAAGTT
IL-6	*Homo sapiens*	TCCTGCAGAAAAAGGCAAAG	GCCCAGTGGACAGGTTTCT
ICAM-1	*Homo sapiens*	GTCCCCCTCAAAAGTCATCC	AACCCCATTCAGCGTCACCT
FZD1	*Homo sapiens*	GAGTTCGTGCCAATCCTGAC	GTCTGTCCATCCTCCCTCTG
PLCE1	*Homo sapiens*	CACGCTCTCAGCTGTCTTGA	TCTCACTGCATTGTAGATCTGGT
KBTBD8	*Homo sapiens*	CATGGACCCCTTCCATGCTT	CCGCTAGTGAACATGGATCTGA
CTRP7	*Homo sapiens*	CAAGTTTTGCCATTTGTGCCAG	GGCAAGCCAGGAATGCTGCAG

Histological examination

The arteries were fixed in 4% paraformaldehyde for one week and then embedded in paraffin. Three round cross-sections (approximately 4 mm in thickness) were cut from the paraffin and stained using a hematoxylin-eosin (HE) staining kit (G1120; Solarbio, Beijing, China), a Masson's trichrome staining kit (G1345; Solarbio) and a Victoria blue staining kit (G1596; Solarbio). Aortic wall thickness, lumen/external diameters and fibrotic areas were measured and analyzed with ImageJ analysis software.

ELISA detection

The plasma samples were collected from rats, and the concentrations of TNF-α and IL-6 were assayed using ELISA kits (Invitrogen) according to the manufacturer's recommendations.

RNA sequencing

Total RNA was extracted from the aortas using Trizol reagent (T9424; Sigma). One microgram of RNA was used to generate the sequencing library. The libraries were sequenced on a BGIseq500 platform (BGI-Shenzhen, Shenzhen, China). For RNA sequencing analysis of the aortas of BK $\alpha^{-/-}$ and WT rats, clean reads were mapped to the rat reference genome using HISAT2 (v2.0.4), and gene expression was calculated using RSEM (v1.2.12). The differentially expressed genes (DEGs) were identified with the criteria of $Q < 0.05$. KEGG enrichment analysis of the annotated DEGs was performed using Phyper (https://en.wikipedia.org/wiki/Hypergeometric_distribution).

GEO datasets and bioinformatics analysis

Gene expression datasets were searched with the keywords *KCNMA1* and aortic; eligible datasets were downloaded from the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) database, and the RNA data were related to the human aortas with reduced *KCNMA1* expression compared with the control group. We found that the GSE 7084 dataset was consistent with the above requirements. This dataset was uploaded to the GPL 570 platform. Then, we used the GEO2R online analysis tool to analyze the genes in the dataset and found the common genes between GSE7084 and the DEGs (fold change > 1.5) in rats. The overlapping genes were validated by qPCR and western blot analysis.

Statistical analysis

GraphPad Prism 8.0 software was used for statistical analysis and statistical graph production. Data are expressed as the mean ± SD. One-way ANOVA followed by Tukey's test was used to compare multiple groups, and the Student's *t* test was used to evaluate the comparison between two groups. $P < 0.05$ was considered statistically significant.

Results

Deletion of the BK channel α subunit induces vascular remodelling in rats

To determine the role of BK channels in vascular remodelling, BK $\alpha^{-/-}$ rats were used in subsequent experiments. BK α protein was not expressed in the aortic tissues of BK $\alpha^{-/-}$ rats (Supplementary Figure S1). The body weight, heart rate and blood pressure of BK $\alpha^{-/-}$ rats were lower than those of WT rats (Supplementary Figures S2 and 3). HE and Masson's trichrome staining showed that the lumen and external diameters of the aortas were reduced (Figure 1A–C), an increase in aortic wall thickness was observed (Figure 1D, E), and aortic fibrosis was increased in BK $\alpha^{-/-}$ rats compared with that in WT rats (Figure 1F,G). Elastin staining showed a disordered elastic lamina in the aortas of BK $\alpha^{-/-}$ rats (Figure 1H). Additionally, we detected the levels of the inflammatory factors interleukin-6 (IL-6) and TNF-α in the plasma of BK $\alpha^{-/-}$ and WT rats by ELISA. The concentrations of IL-6 and TNF-α were increased in the plasma of BK $\alpha^{-/-}$ rats compared with those of WT rats (Supplementary Figure S4). Overall, these results revealed that deletion of BK α contributed to vascular remodelling in rats.

The deletion and functional inhibition of BK α in HUASMCs increases vascular remodelling-related markers

The enhanced levels of MMPs and inflammatory cytokines and the reduced expressions of SMC contractile proteins contribute to

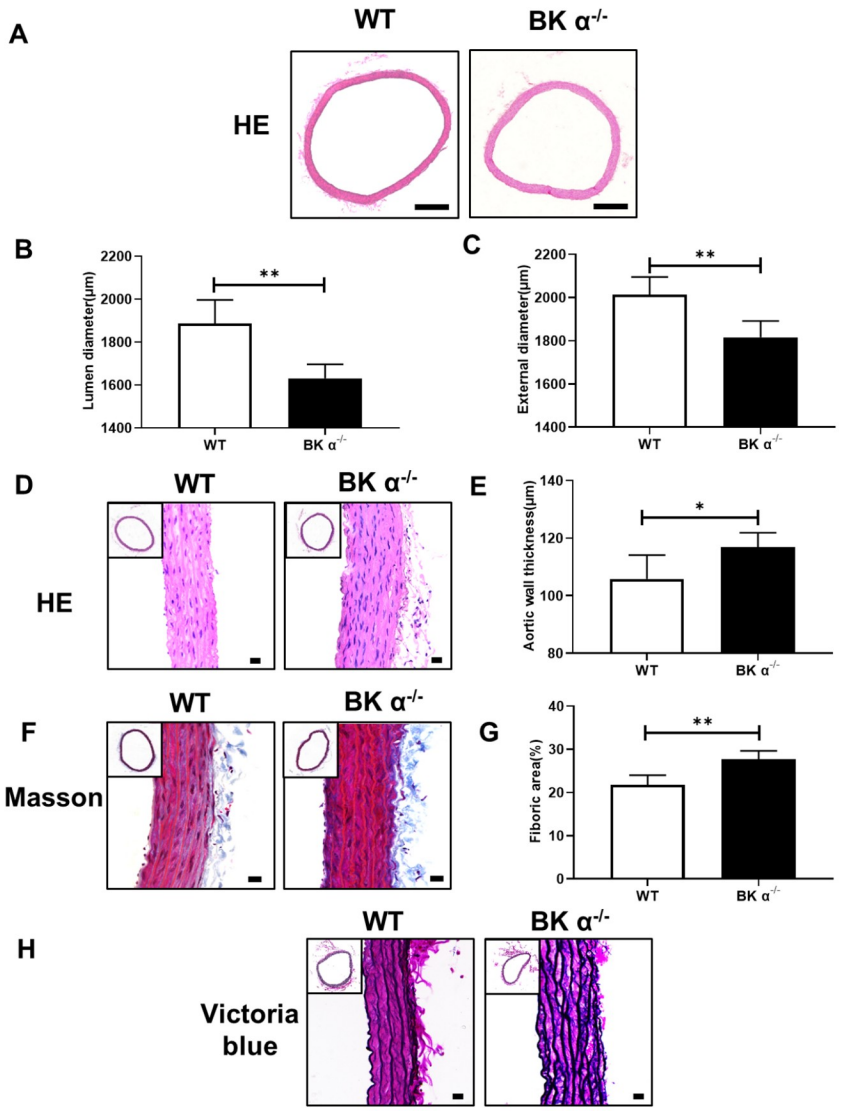

Figure 1. The aortas of BK $\alpha^{-/-}$ rats exhibited vascular remodeling (A) Representative images of HE staining and statistical diagrams of the lumen diameter (B) and the external diameter (C) of the aortas in 6-month-old WT and BK $\alpha^{-/-}$ rats. Scale bar: 500 μm. (D) Representative images of HE staining and statistical diagrams of the aortic wall thickness (E) in 6-month-old WT and BK $\alpha^{-/-}$ rats. Scale bar: 20 μm. (F,G) Representative images of Masson's trichrome staining and statistical diagrams of the fibrotic area of the aorta in 6-month-old WT and BK $\alpha^{-/-}$ rats. Scale bar: 20 μm. (H) Representative images of elastin staining (Victoria blue) in the aortas of 6-month-old WT and BK $\alpha^{-/-}$ rats. Scale bar: 20 μm. Data are shown as the mean ± SD, $n = 5$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$.

vascular remodelling. Therefore, we investigated the effect of BK α on the expression of these factors in HUASMCs. Two different approaches were employed. First, we performed an RNA interference experiment (Supplementary Figure S5). The data showed that the expressions of the SMC contractile phenotype proteins SM-22α and Calponin were decreased (Figure 2A), an increase in MMP2 and MMP9 was observed (Figure 2B–D), and the expression of IL-6 was upregulated by BK α knockdown (Figure 2E,F). Second, to obtain more evidence supporting the involvement of BK α in the inhibition of vascular remodelling, the BK channel inhibitor paxilline was used *in vitro*. The data were similar to the RNA interference results (Figure 3). These results suggested that the deletion and functional inhibition of BK α could promote vascular remodelling by increasing the release of inflammatory factors and MMPs and reducing the expressions of SMC contractile phenotype proteins.

CTRP7 is a novel downstream target gene of BK α in HUASMCs

To identify the downstream target genes of BK α, aortas were obtained from BK $\alpha^{-/-}$ and WT rats and used for RNA sequencing. Transcriptome analysis showed 375 differentially expressed genes (DEGs) between BK $\alpha^{-/-}$ and WT rats ($P < 0.05$), of which 171 genes were upregulated and 204 genes were downregulated. Then, homologous comparison with human genes was performed, and 164 DEGs were selected in rats ($P < 0.05$, fold change > 1.5). Ten common genes overlapped between the 164 previously selected genes and DEGs in the GEO database GSE 7084 (Figure 4A,B):

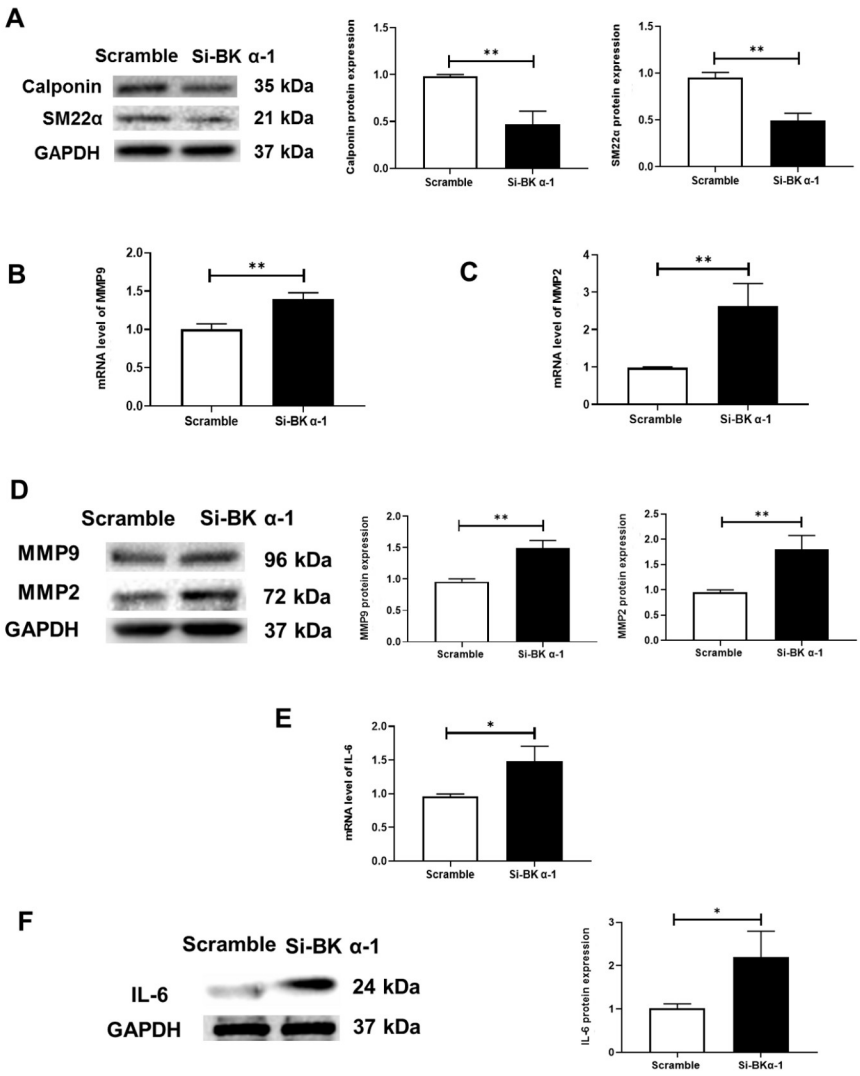

Figure 2. Expressions of inflammatory cytokines, MMPs and SMC contractile phenotype proteins *in vitro* after BK α knockdown (A) Representative blots and statistical diagrams showing the expressions of Calponin and SM22α, as determined by western blot analysis *in vitro* after BK α knockdown. (B–D) Representative blots and statistical diagrams showing the expressions of MMP9 and MMP2, as determined by western blot analysis and qPCR *in vitro* after BK α knockdown. (E,F) Representative blots and statistical diagrams showing the expression of IL-6, as determined by western blot analysis and qPCR *in vitro* after BK α knockdown. Data are shown as the mean ± SD, $n = 3$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$.

KCNMA1, *FZD1*, *C1qtnf7*, *MYOZ2*, *HSPB7*, *PLCE1*, *POSTN*, *STK38L*, *KBTBD8*, and *TGFBI* (Supplementary Table S1). Among them, *KBTBD8*, *FZD1*, *C1qtnf7*, *PLCE1*, and *KCNMA1* had the same trend in the transcriptome analysis and GSE7084 dataset. Next, except for *KCNMA1* (BK α), four genes were verified by qPCR (Figure 4C,D). Among them, *C1qtnf7* (CTRP7) was the most significantly altered gene *in vivo* and *in vitro*. Furthermore, western blot analysis revealed that the trend of CTRP7 expression in the aortas of BK $\alpha^{-/-}$ rats and HUASMCs with *BK α* knockdown was consistent with that in the transcriptome analysis (Figure 4E,F). Based on these results, we attempted to explore the role of CTRP7 in vascular remodelling.

CTRP7 is critically involved in BK α deficiency-mediated vascular remodelling in HUASMCs

To determine the role of CTRP7 in BK α deficiency-mediated vascular remodelling, RNA interference technique was used to decrease the level of CTRP7 in HUASMCs (Supplementary Figure S6). The data showed that decreased expressions of SMC contractile phenotype proteins were observed after *CTRP7* knockdown (Figure 5A). Simultaneously, the inhibitory effect on the mRNA and protein levels of IL-6 and ICAM-1 mRNA disappeared after *CTRP7* knockdown (Figure 5B–D). However, CTRP7 deficiency reduced the mRNA levels of MMP2 and MMP9 (Supplementary Figure S7). Taken together, these results suggested that CTRP7 was an important mediator of vascular smooth muscle cell (VSMC) protection.

The PI3K/Akt pathway is downstream of BK α/CTRP7 deficiency-mediated vascular remodelling in HUASMCs

Having demonstrated that BK α and CTRP7 are involved in VSMC protection, we attempted to explore the molecular signaling

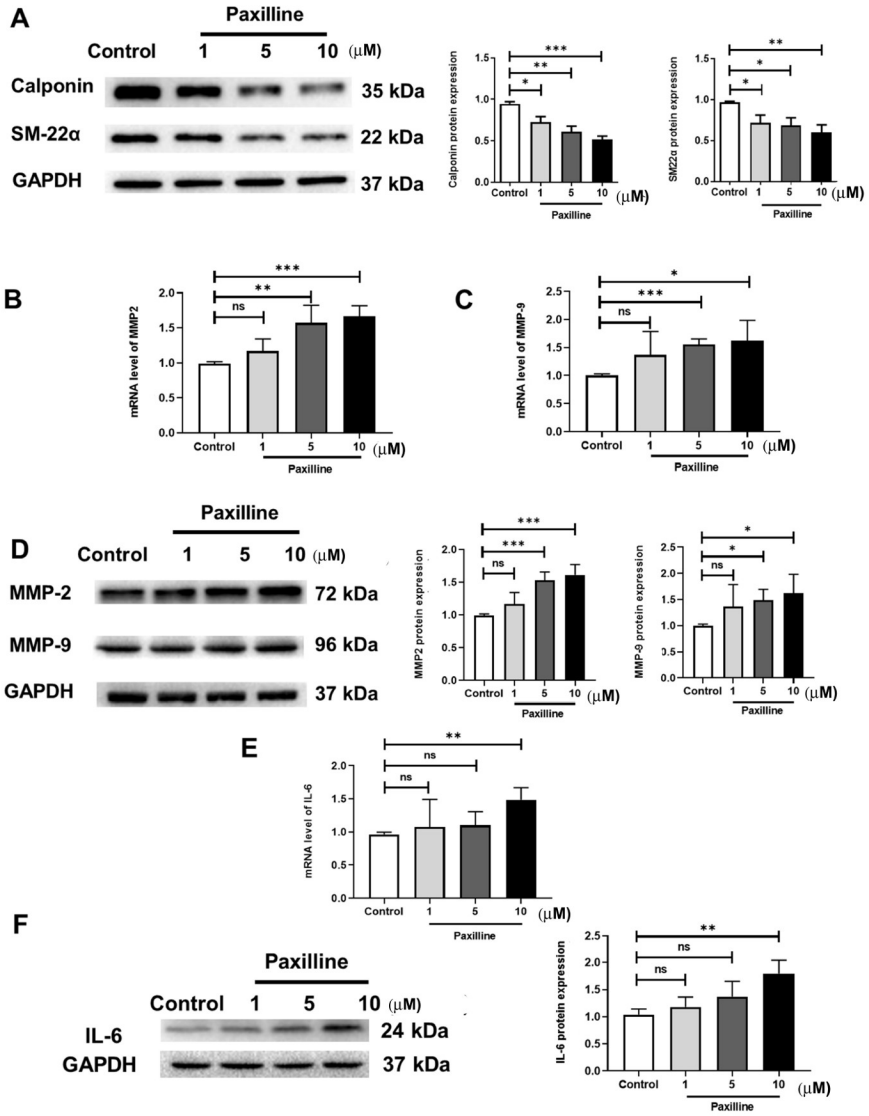

Figure 3. Expressions of inflammatory cytokines, MMPs and SMC contractile phenotype proteins *in vitro* after paxilline treatment (A) Representative blots and statistical diagrams showing the *in vitro* expressions of Calponin and SM22-α, as determined by western blot analysis after treatment with paxilline for 72 h. (B–D) Representative blots and statistical diagrams showing the expressions of MMP9 and MMP2 *in vitro* after treatment with paxilline, as determined by western blot analysis (72 h) and qPCR (24 h). (E,F) Representative blots and statistical diagrams showing the expression of IL-6 *in vitro* after paxilline treatment, as determined by western blot analysis (72 h) and qPCR (24 h). Data are shown as the mean ± SD, $n = 3$ per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns, not significant.

pathways responsible for these effects. First, pathway analysis of the upregulated DEGs was conducted to identify the signaling pathways that were most likely responsible for BK α-mediated vascular protection. The results revealed that the focal adhesion pathway, the PI3K/Akt signaling pathway, and the TGF-β signaling pathway exhibited significant enrichment (Figure 6A; $P < 0.05$). We next examined these three signaling pathways in the context of BK α deficiency. Western blot analysis showed that only the PI3K/Akt signaling pathway was upregulated by *BK α* knockdown (Figure 6B). Furthermore, CTRP7 deficiency activated the PI3K/Akt signaling pathway (Figure 6C). These results suggested that the PI3K/Akt pathway was the downstream signaling pathway activated by BK α/CTRP7 deficiency signaling.

Discussion

In the present study, we first report that the deletion of BK α promotes vascular inflammation and decreases SMC contractile phenotype proteins, thereby leading to vascular remodelling. Furthermore, this study provides evidence that CTRP7 is expressed in vascular smooth muscle and that BK deficiency activates the PI3K/Akt signaling pathway by reducing CTRP7, which results in vascular disease.

BK channels are widely distributed in the cardiovascular system. These channels are ubiquitously expressed, especially in VSMCs, and have large single channel conductance values [12]. In VSMCs, BK channels are composed of pore-forming α subunits and auxiliary subunits (β subunits and γ subunits) [13]. The α subunits regulate the concentrations of Ca^{2+} and K^{+} inside and outside the cell and vascular tension. The increase in intracellular Ca^{2+} concentration or

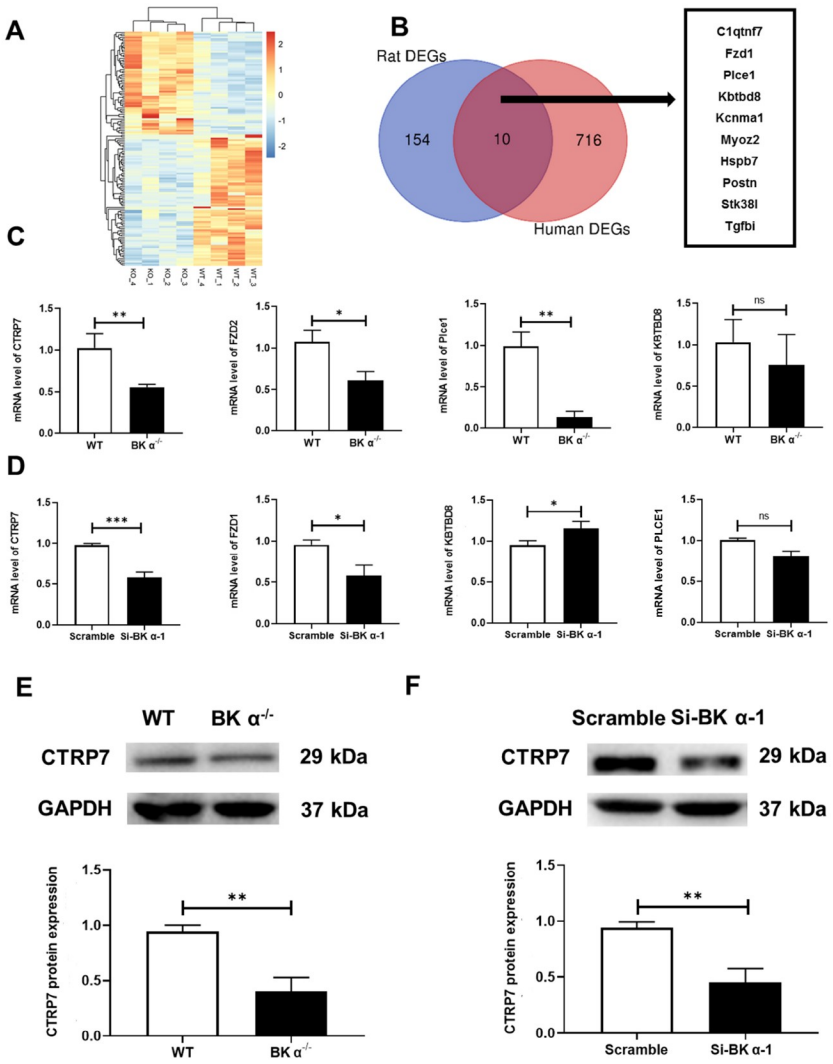

Figure 4. ***CTRP7*** **is a novel downstream target gene of BK α** (A) Heatmap showing the DEGs from the transcriptome analysis. (B) Venn diagram showing the number of overlapping DEGs from the transcriptome analysis (fold change > 1.5) and the GSE 7084 dataset. (C) Representative statistical diagrams showing the expressions of KBTBD8, PLCE1, FZD1, and CTRP7 determined by qPCR in the aortas of WT and BK $\alpha^{-/-}$ rats, $n = 4$ per group. (D) Representative statistical diagrams showing the expressions of *KBTBD8*, *PLCE1*, *FZD1*, and *CTRP7* determined by qPCR in HUASMCs after BK α knockdown. $n = 3$ per group. (E,F) Representative blots and statistical diagrams showing the expression of CTRP7 in the aortas of WT and BK $\alpha^{-/-}$ rats and HUASMCs after BK α knockdown, determined by western blot analysis. $n = 4$ per group. Data are shown as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant.

the change in voltage activates the BK channel so that the intracellular K^+ flows out through the pore of the channel composed of the α subunit, thereby dilating blood vessels and maintaining a balance of vascular tension [14]. Auxiliary subunits modulate the activity and opening frequency of BK channels in voltage- and calcium-dependent manners [14]. A previous study showed that blood pressure was significantly increased in β subunit knockout mice [15]. Another study showed that BKβ1 subunit-knockout mice fed with a high-fat diet had significant increases in vascular fibrosis and remodelling [16], but evidence for the effect of α subunits on vascular remodelling is lacking. In this study, we found that the blood vessels of BK $\alpha^{-/-}$ rats spontaneously developed vascular remodelling, including thickening of the vessel wall, increased deposition of collagen fibers, and disordered arrangement of elastic fibers. These results indicated that the BK channel α subunit might play a crucial role in vascular remodelling.

Interestingly, it was reported that blood pressure was increased in BK $\alpha^{-/-}$ mice [17]; however in the present study we found that the blood pressure and heart rate were deceased in BK $\alpha^{-/-}$ rats. The reason for this discrepancy may be that different experimental animal species were used, i.e., we used rats and they used mice. Second, blood pressure is determined by cardiac ejection, blood volume, and vascular resistance [18]. In our previous studies, compared with WT rats, BK $\alpha^{-/-}$ rats showed enhanced vasoconstriction; however, the left ventricular wall became thinner, and the myocardial area was smaller. At the same time, there was a decrease in left ventricular systolic function (the left ventricular ejection fraction and left ventricular short-axis shortening rate were decreased) in BK $\alpha^{-/-}$ rats [8]. In addition, it was reported that blood pressure was reduced in BK channel cardiac conditional knockout

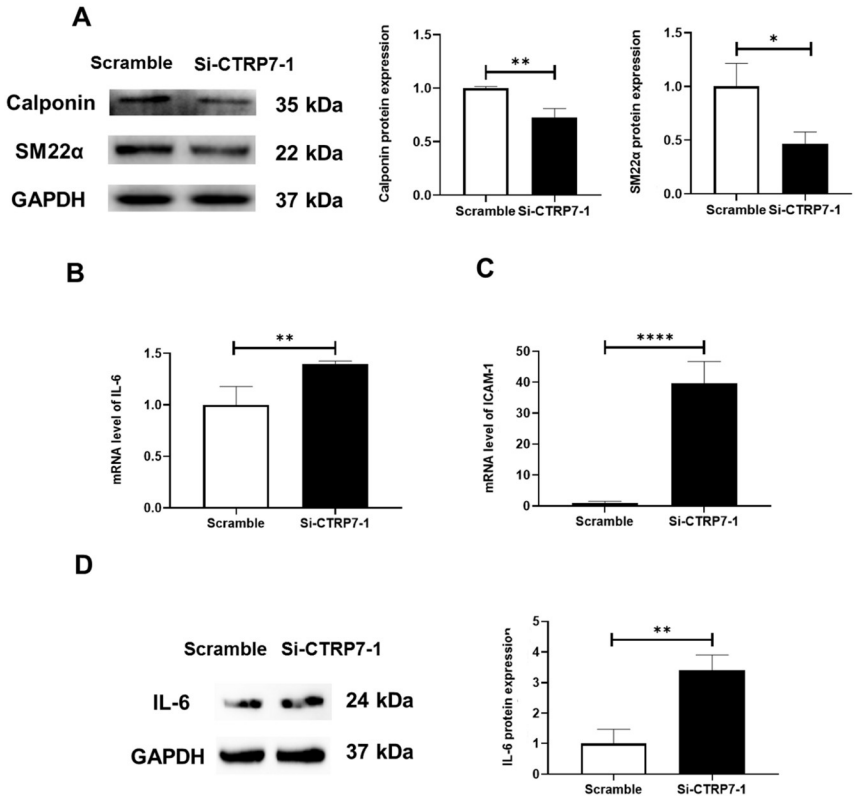

Figure 5. Expressions of SMC contractile phenotype proteins and inflammatory cytokines in HUASMCs after *CTRP7* knockdown (A) Representative blots and statistical diagrams showing the expressions of Calponin and SM22-α in HUASMCs after *CTRP7* knockdown, as determined by western blot analysis. (B–D) Representative blots and statistical diagrams showing the expression of IL-6, as determined by western blot analysis and qPCR in HUASMCs after *CTRP7* knockdown. Data are shown as the mean ± SD, $n = 3$ per group. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$.

mice [19]. Therefore, the reason for this discrepancy may be that whole-body BK $\alpha^{-/-}$ rats showed various phenotypic disorders that may affect blood pressure, such as reduced cardiac function [8]. Apparently, the data are significant because they suggest that vascular BK channels protect against vascular remodelling and fibrosis via blood pressure-independent mechanisms.

After stimulation of inflammation and other factors, the levels of MMPs in VSMCs and macrophages were markedly increased [20]. MMPs are powerful metalloproteinases that degrade the extracellular matrix, including elastic fibers and collagen fibers, and stimulate SMC proliferation and migration [6,21]. In the present study, we demonstrated for the first time that there was an increase in MMPs after the inhibition of BK α expression and activity in VSMCs. These results suggested that BK channels might have a beneficial effect on cardiovascular protection.

Abnormal expression or weakened activity of BK channels has a significant impact on the human body. Abnormal expression of BK channels, such as BK channel phosphorylation, may induce channel activity [12]. Furthermore, the α subunit can also bind to other proteins, such as focal adhesion kinase (FAK) and the protein kinase A (PKA) complex [22], and is associated with cell signaling events [1]. Therefore, we inhibited the expression and activity of BK channels *in vitro* to observe their effect on VSMCs. Our findings showed that inhibition of α subunit expression or function impaired VSMCs. This observation provides clues that BK channels are potential targets for the precise treatment of vascular diseases.

CTRP7 also shows a certain protective effect on VSMCs. CTRP7, which is known as C1q tumor necrosis factor-related protein 7, belongs to the complement C1q tumor necrosis factor-related protein superfamily [23]. CTRP proteins are structurally similar to adiponectin and consist of an N-terminal signal peptide, a short variable region, a collagen domain, and a C-terminal globular domain that is homologous to complement component 1q [24]. In addition, CTRPs have many functions similar to those of adiponectin, including lipid regulation, insulin sensitization and anti-inflammatory effects. The difference is that adiponectin is secreted by adipose tissue, while CTRP proteins are widely distributed in the body [25]. CTRP family members play an important role in the protection of cardiovascular disease and the regulation of inflammation. For example, CTRP1 prevents the development of pathological vascular remodelling [26,27]. CTRP3 can protect cardiac function by promoting angiogenesis, and inhibiting apoptosis and fibrosis [28]. CTRP6 can inhibit postinfarction cardiac fibrosis by inhibiting fibroblast migration and transition to myofibroblasts [29]. CTRP9 is expressed at high level in the heart, and recent studies have shown that it can attenuate acute ischaemia-reperfusion injury and reduce ventricular remodelling after myocardial infarction [30] and it also has certain vasodilatory functions [31]. However, other functions of these CTRP family members are largely unknown.

At present, the function of CTRP7 is not clearly understood. In one report, the deletion of CTRP7 reduced obesity-related glucose intolerance, adipose tissue inflammation, and hepatic stress [32]. A

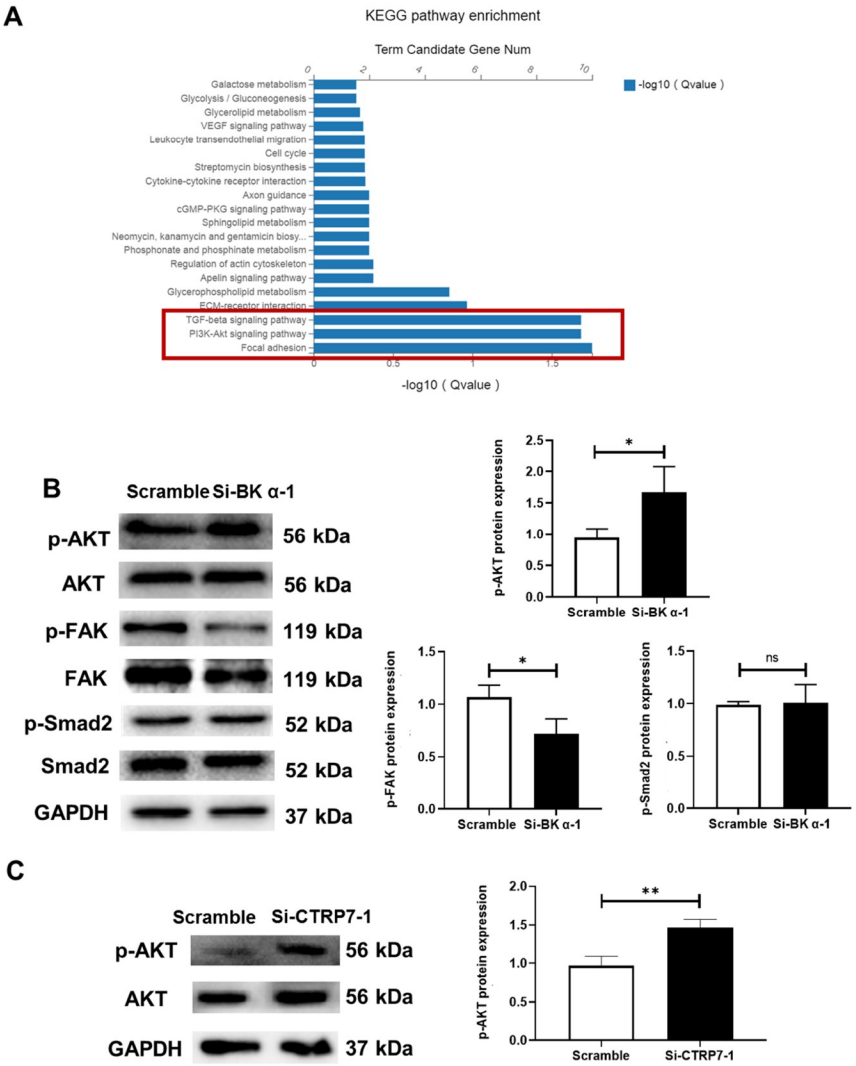

Figure 6. Effect of BK α deficiency on downstream signaling in the aortas of rats and HUASMCs (A) Representative graphs showing the top enriched signaling pathways of DEGs in the aortas of WT and BK $\alpha^{-/-}$ rats, as revealed by KEGG pathway analysis. (B) Representative blots and statistical diagrams showing the expressions of p-Akt, p-FAK, and p-Smad2 in HUASMCs after BK α knockdown, as determined by western blot analysis. (C) Representative blots and statistical diagrams showing the expression of p-Akt in HUASMCs after CTRP7 knockdown, as determined by western blot analysis. Data are shown as the mean ± SD, $n = 3$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$, ns, not significant.

recent study showed that circulating CTRP7 is a potential predictor of metabolic syndrome [33]. Metabolic syndrome is often accompanied by chronic inflammation [34], and elevated expression of CTRP7 may be associated with inflammation. However, we observed that CTRP7 deficiency contributed to inflammation in VSMCs. This finding seemed to contradict the proinflammatory effect of CTRP7 reported in related studies [32]. Of note, the contradiction may be attributed to different pathological models. Although there are few studies on CTRP7, multiple studies have shown that adiponectin, a homologue of the CTRP family, plays different roles in different diseases [35]. CTRP7, as a paralogue of adiponectin, may play a similar role to adiponectin, which indicates that the role of CTRP7 is complex. Another report mentioned that circulating CTRP7 level in coronary artery disease (CAD) patients was reduced [36], suggesting that CTRP7 is a potential biomarker for CAD diagnosis. We showed for the first time that CTRP7 was expressed in smooth muscle and that CTRP7 deficiency inhibited SMC contractile phenotype protein expression. These data together revealed that CTRP7 plays vital physiological and pathological roles in cardiovascular disease and may have a certain protective effect on the cardiovascular system. Considering the importance of CTRP7 in the human body, it deserves to be explored more deeply.

The PI3K/Akt pathway acts as a critical regulator of cell proliferation and nutrient metabolism. It has been shown that activation of the PI3K/Akt pathway is closely associated with vascular remodelling [37]. Notably, our study revealed that the reduced expressions of BK α and CTRP7 mediated the activation of the PI3K/Akt signaling pathway. These data identified the downstream pathway of BK α/CTRP7 deficiency-mediated vascular remodelling. Since we have only performed preliminary studies on CTRP7, the specific contribution of CTRP7 in VSMCs to vascular

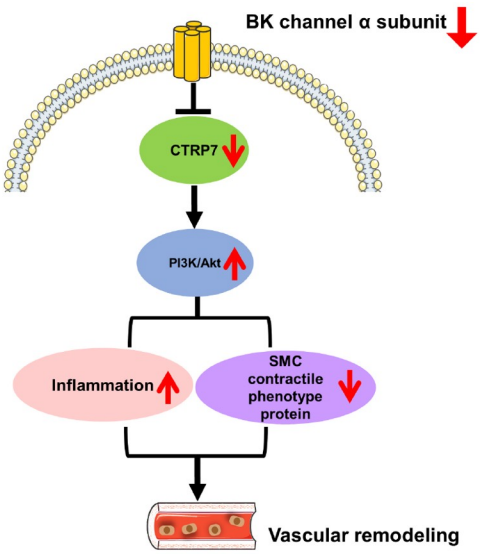

Figure 7. Schematic illustration of the process of vascular remodelling induced by BK channels SMC, smooth muscle cell; CTRP7, C1q/tumor necrosis factor -related protein 7.

remodelling needs more in-depth research.

It is well known that the role of BK channels is closely related to Ca^{2+}. One study showed that there was no significant change in the intracellular Ca^{2+} concentration of vascular smooth muscle cells from BK $\alpha^{-/-}$ mice compared with that from WT mice [17]. The CTRPs are paralogues of adiponectin, and their functions are similar [25]. A previous study showed that high-density lipoprotein can promote adiponectin expression through the Ca^{2+}/calmodulin (CaM)-dependent protein kinase IV (CaMKIV) cascade [38]. However, the relationship between CTRP7 and Ca^{2+} has not been reported. In future studies, we can measure the Ca^{2+} concentration in vascular smooth muscle cells of BK $\alpha^{-/-}$ rats and study the relationship between CTRP7 and Ca^{2+}.

In summary, we found that loss of BK channels inhibits the expressions of SMC contractile phenotype proteins and promotes the massive release of inflammatory factors, which may lead to vascular remodeling (Figure 7). In addition, we first observed that CTRP7 is distributed in VSMCs and CTRP7 may have anti-inflammatory and other protective effects on VSMCs, suggesting that BK channels and CTRP7 may be potential targets for the prevention of vascular remodelling and other vascular diseases and for drug development.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 91539205 and 81900415) and the Beijing Natural Science Foundation Program and Scientific Research Key Program of Beijing Municipal Commission of Education (No. KZ201810025039).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Toro L, Li M, Zhang Z, Singh H, Wu Y, Stefani E. MaxiK channel and cell signalling. *Pflugers Arch - Eur J Physiol* 2014, 466: 875–886
2. Sancho M, Kyle BD. The large-conductance, calcium-activated potassium channel: a big key regulator of cell physiology. *Front Physiol* 2021, 12: 750615
3. Yang Y, Li PY, Cheng J, Mao L, Wen J, Tan XQ, Liu ZF, *et al.* Function of BK_{Ca} channels is reduced in human vascular smooth muscle cells from Han Chinese patients with hypertension. *Hypertension* 2013, 61: 519–525
4. Tobal R, Potjewijd J, Empel VP, Ysermans R, Schurgers LJ, Reuteling-sperger CP, Damoiseaux JG, *et al.* Vascular remodeling in pulmonary arterial hypertension: the potential involvement of innate and adaptive immunity. *Front Med* 2021, 8: 806899
5. Castorena-Gonzalez JA, Staiculescu MC, Foote C, Martinez-Lemus LA. Mechanisms of the inward remodeling process in resistance vessels: is the actin cytoskeleton involved? *Microcirculation* 2014, 21: 219–229
6. Jaminon A, Reesink K, Kroon A, Schurgers L. The role of vascular smooth muscle cells in arterial Remodeling: focus on calcification-related processes. *Int J Mol Sci* 2019, 20: 5694
7. Whiteford JR, De Rossi G, Woodfin A. Mutually supportive mechanisms of inflammation and vascular Remodelingr. *Int Rev Cell Mol Biol* 2016, 326: 201–278
8. He C, Li X, Wang M, Zhang S, Liu H. Deletion of BK channels decreased skeletal and cardiac muscle function but increased smooth muscle contraction in rats. *Biochem Biophys Res Commun* 2021, 570: 8–14
9. Xu H, Wang Y, Garver H, Galligan JJ, Fink GD. Vascular BK channel deficiency exacerbates organ damage and mortality in endotoxemic mice. *J Cardiovasc Pharmacol* 2012, 59: 207–214
10. Wan XJ, Zhao HC, Zhang P, Huo B, Shen BR, Yan ZQ, Qi YX, *et al.* Involvement of BK channel in differentiation of vascular smooth muscle cells induced by mechanical stretch. *Int J Biochem Cell Biol* 2015, 59: 21–29
11. Ren J, Cheng Y, Wen X, Liu P, Zhao F, Xin F, Wang M, *et al.* BK_{Ca} channel participates in insulin-induced lipid deposition in adipocytes by increasing intracellular calcium. *J Cell Physiol* 2021, 236: 5818–5831
12. Krishnamoorthy-Natarajan G, Koide M. BK channels in the vascular system. *Int Rev Neurobiol* 2016, 128: 401–438
13. Carvalho-de-Souza JL, Varanda WA, Tostes RC, Chignalia AZ. BK channels in cardiovascular diseases and aging. *Aging Dis* 2013, 4: 38–49
14. Dopico AM, Bukiya AN, Jaggar JH. Calcium- and voltage-gated BK channels in vascular smooth muscle. *Pflugers Arch - Eur J Physiol* 2018, 470: 1271–1289
15. Pluger S, Faulhaber J, Furstenau M, Lohn M, Waldschutz R, Gollasch M, Haller H, *et al.* Mice with disrupted BK channel β1 subunit gene feature abnormal Ca^{2+} spark/STOC coupling and elevated blood pressure. *Circ Res* 2000, 87: 53–60
16. Xu H, Garver H, Fernandes R, Phelps JT, Harkema JJ, Galligan JJ, Fink GD. BK channel β1-subunit deficiency exacerbates vascular fibrosis and remodelling but does not promote hypertension in high-fat fed obesity in mice. *J Hypertension* 2015, 33: 1611–1623
17. Sausbier M, Arntz C, Bucurenciu I, Zhao H, Zhou XB, Sausbier U, Feil S, *et al.* Elevated blood pressure linked to primary hyperaldosteronism and impaired vasodilation in BK channel–deficient mice. *Circulation* 2005, 112: 60–68
18. Mullington JM, Haack M, Toth M, Serrador JM, Meier-Ewert HK. Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation. *Prog Cardiovasc Dis* 2009, 51: 294–302
19. Frankenreiter S, Bednarczyk P, Kniess A, Bork NI, Straubinger J, Koprowski P, Wrzosek A, *et al.* cGMP-elevating compounds and ischemic

conditioning provide cardioprotection against ischemia and reperfusion injury via cardiomyocyte-specific BK channels. *Circulation* 2017, 136: 2337–2355
20. Cevik C, Otahbachi M, Nugent K, Warangkana C, Meyerrose G. Effect of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition on serum matrix metalloproteinase-13 and tissue inhibitor matrix metalloproteinase-1 levels as a sign of plaque stabilization. *J Cardiovasc Med* 2008, 9: 1274–1278
21. Ma Z, Mao C, Jia Y, Fu Y, Kong W. Extracellular matrix dynamics in vascular remodeling. *Am J Physiol-Cell Physiol* 2020, 319: C481–C499
22. Tian L, Coghill LS, MacDonald SHF, Armstrong DL, Shipston MJ. Leucine zipper domain targets cAMP-dependent protein kinase to mammalian BK channels. *J Biol Chem* 2003, 278: 8669–8677
23. Kong M, Gao Y, Guo X, Xie Y, Yu Y. Role of the CTRP family in tumor development and progression. *Oncol Lett* 2021, 22: 723
24. Ressl S, Vu BK, Vivona S, Martinelli DC, Südhof TC, Brunger AT. Structures of C1q-like proteins reveal unique features among the C1q/TNF superfamily. *Structure* 2015, 23: 688–699
25. Li L, Aslam M, Siegler BH, Niemann B, Rohrbach S. Comparative analysis of CTRP-mediated effects on cardiomyocyte glucose metabolism: cross talk between AMPK and Akt signaling pathway. *Cells* 2021, 10: 905
26. Kanemura N, Shibata R, Ohashi K, Ogawa H, Hiramatsu-Ito M, Enomoto T, Yuasa D, ***et al.*** C1q/TNF-related protein 1 prevents neointimal formation after arterial injury. *Atherosclerosis* 2017, 257: 138–145
27. Shen Y, Lu L, Liu ZH, Wu F, Zhu JZ, Sun Z, Zhang RY, ***et al.*** Increased serum level of CTRP1 is associated with low coronary collateralization in stable angina patients with chronic total occlusion. *Int J Cardiol* 2014, 174: 203–206
28. Wu D, Lei H, Wang JY, Zhang CL, Feng H, Fu FY, Li L, ***et al.*** CTRP3 attenuates post-infarct cardiac fibrosis by targeting Smad3 activation and inhibiting myofibroblast differentiation. *J Mol Med* 2015, 93: 1311–1325
29. Lei H, Wu D, Wang JY, Li L, Zhang CL, Feng H, Fu FY, ***et al.*** C1q/tumor necrosis factor-related protein-6 attenuates post-infarct cardiac fibrosis by targeting RhoA/MRTF-A pathway and inhibiting myofibroblast differentiation. *Basic Res Cardiol* 2015, 110: 35
30. Sun Y, Yi W, Yuan Y, Lau WB, Yi D, Wang X, Wang Y, ***et al.*** C1q/tumor necrosis factor–related protein-9, a novel adipocyte-derived cytokine, attenuates adverse remodeling in the ischemic mouse heart via protein kinase A activation. *Circulation* 2013, 128
31. Zheng Q, Yuan Y, Yi W, Lau WB, Wang Y, Wang X, Sun Y, ***et al.*** C1q/TNF-related proteins, a family of novel adipokines, induce vascular relaxation through the adiponectin receptor-1/AMPK/eNOS/nitric oxide signaling pathway. *Arterioscler Thromb Vasc Biol* 2011, 31: 2616–2623
32. Petersen PS, Lei X, Wolf RM, Rodriguez S, Tan SY, Little HC, Schweitzer MA, ***et al.*** CTRP7 deletion attenuates obesity-linked glucose intolerance, adipose tissue inflammation, and hepatic stress. *Am J Physiol-Endocrinol Metab* 2017, 312: E309–E325
33. Hu W, Zhan B, Li Q, Yang G, Yang M, Tan M, Geng S, ***et al.*** Circulating CTRP7 is a potential predictor for metabolic syndrome. *Front Endocrinol* 2021, 12: 774309
34. McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. *Clin Dermatol* 2018, 36: 14–20
35. Jang AY, Scherer PE, Kim JY, Lim S, Koh KK. Adiponectin and cardiometabolic trait and mortality: where do we go? *Cardiovasc Res* 2022, 118: 2074–2084
36. Zhang Y, Liu C, Liu J, Guo R, Yan Z, Liu W, Lau WB, ***et al.*** Implications of C1q/TNF-related protein superfamily in patients with coronary artery disease. *Sci Rep* 2020, 10: 878
37. Liu QF, Yu HW, Sun LL, You L, Tao GZ, Qu BZ. Apelin-13 upregulates Egr-1 expression in rat vascular smooth muscle cells through the PI3K/Akt and PKC signaling pathways. *Biochem Biophys Res Commun* 2015, 468: 617–621
38. Kobayashi T, Imachi H, Fukunaga K, Lyu J, Sato S, Saheki T, Ibata T, ***et al.*** HDL promotes adiponectin gene expression via the CAMKK/CAMKIV pathway. *J Mol Endocrinol* 2022, 68: 89–98