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Chronic central miR-29b antagonism alleviates angiotensin II-induced hypertension and vascular endothelial dysfunction

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ABSTRACT

Dysregulation of miR-29 has been revealed in multiple diseases, but its role in the development of hypertension and vascular endothelial dysfunction has not been defined. Here, we found that, compared with the wild-type (WT) Wistar rats, miR-29b was robustly upregulated in spontaneously hypertensive rats (SHRs), while CTRP6 was distinctly downregulated. There were two miRNA-responding-elements (MREs) for miR-29 in the 3′-UTR of CTRP6 mRNA, and the luciferase activity assay revealed that miR-29b directly targeted CTRP6 mRNA. Intraventricular injection was applied to deliver the miR-29b mimic or miR-29b inhibitor (4 mg/kg) into SHRs once two weeks from 10th week. Downregulation of miR-29b could increase serum CTRP6 content in SHRs, decrease the arterial systolic pressure, reduce serum concentrations of Ang II and ET-1, and enhance serum NO content. Meanwhile, we demonstrated that inhibition of miR-29b increased the phosphorylation of ERK1/2 to activate PPARγ, an inducer of Ang II. Finally, miR-29b expression was manipulated in, and CTRP6 recombinant protein was applied to incubate with the primary aortic endothelial cells. Inhibition of miR-29b increased CTRP6 expression, improved cell proliferation and migration, suppressed secretion of Ang II and ET-1, and decreased ROS accumulation and LDH release, displaying a similar effect to the CTRP6 recombinant protein. Moreover, the CTRP6 recombinant protein could antagonize the suppressive effect of miR-29b on activation of the ERK/PPARγ axis and function of aortic endothelial cells. In conclusion, miR-29b antagonism can alleviate Ang II-induced hypertension and vascular endothelial dysfunction through activating the CTRP6/ERK/PPARγ axis.

1. Introduction

Hypertension, a common chronic illness with uncertain etiology, is regarded as one of the important risk factors for acute cardio- and cerebrovascular diseases [1]. According to the latest survey, hypertension has become a notable health problem that affects approximately 30% of Chinese adults [2]. Although most current drugs have been adapted to controlling blood pressure, but most of them are far from effective for lowering the incidence of complications. Angiotensin II (Ang II) is the most essential member of the angiotensin family and an important effector in the rein-angiotensin system. Ang II is also acknowledged as a vital mediator of hypertension and vascular endothelial dysfunction [3]. In fact, it has become evident that Ang II not only enhances the blood pressure resulting in contraction through stimulating the G protein and an IP3-dependent mechanism, but also induces vascular inflammation and endothelial dysfunction by activating the secretion of pro-inflammatory cytokines [4,5]. Given the significant role of Ang II in the hypertension and endothelial vascular dysfunction, it is of importance to explore novel therapeutic targets to reverse Ang II-induced pathological progressions.

MicroRNAs (miRNAs) are a group of small non-coding RNAs with length of 18–25 nucleotides [6]. Accumulated studies have shown that dysregulation of miRNAs have been implicated in a variety of biological processes and they can be regarded as diagnostic and therapeutic markers in many diseases [7]. The miR-29 family contains three largely homologous members (miR-29a, miR-29b and miR-29c) and is differentially expressed in particular diseases [8]. Among these subtypes, miR-29b was revealed as a potential circulating biomarker for stroke outcomes prediction. Upregulation of miR-29b could reduce blood brain barrier disruption after ischemic stroke [9]. MiR-29b has been also shown to inhibit Ang II-induced cardiac fibrosis, indicating a protective role of miR-29b in normal function of the heart in response to Ang II [10]. However, a couple of recent studies revealed that miR-29 family were significantly upregulated in patients with hypertension and positively correlated with their blood pressure, suggesting a different role of miR-29 in pathogenesis of hypertension [11,12]. Therefore, it

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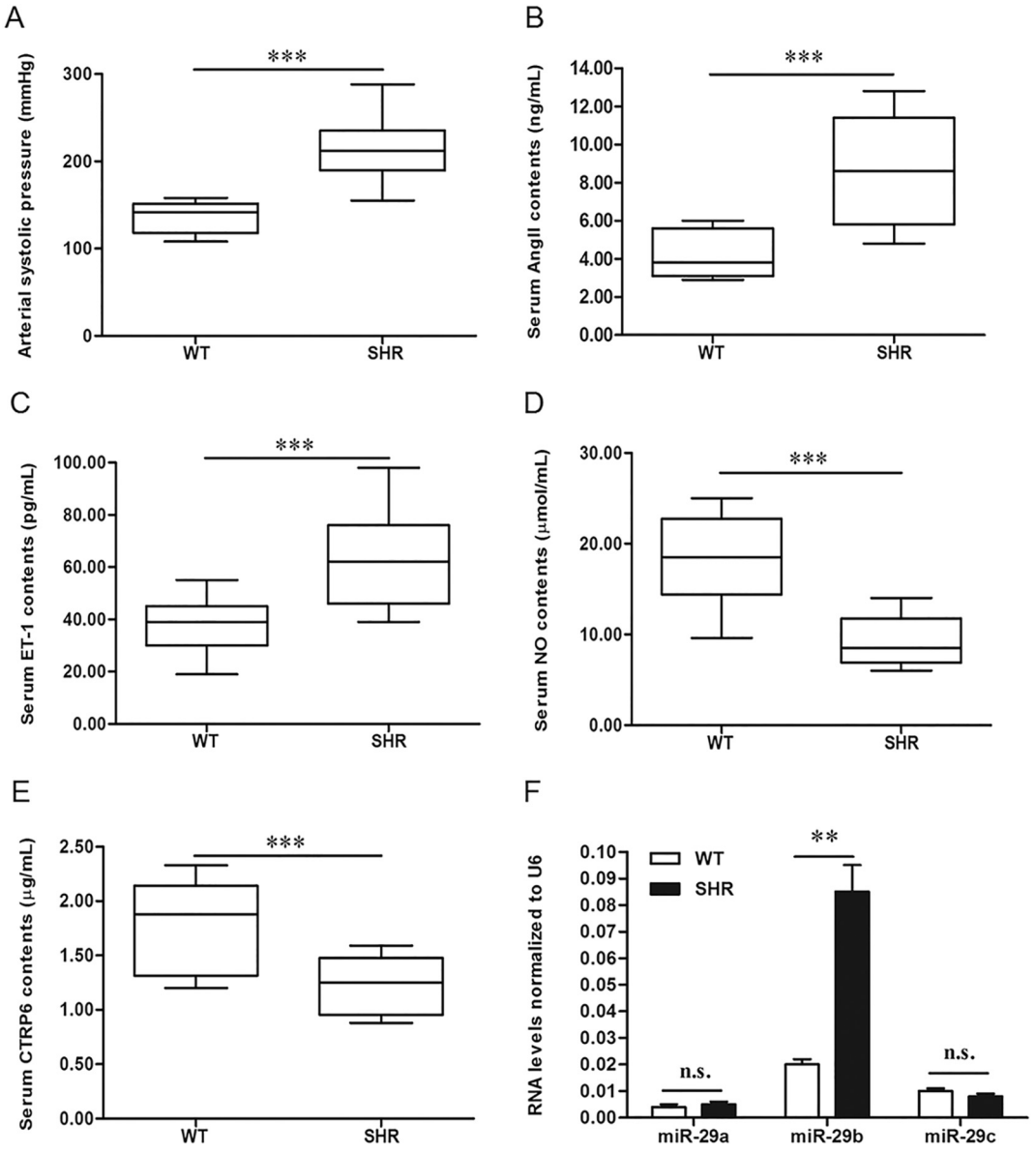

Fig. 1. Expression of CTRP6 and miR-29 were measured in SHRs. Serum samples and aortic samples were respectively extracted from 20 WT rats and 15 SHRs. (A) Comparison of the arterial systolic pressures between WT rats and SHRs. (B–E) Serum concentrations of Ang II, ET-1, NO and CTRP6 were tested in WT rats and SHRs using ELISA kits. (F) Levels of miR-29 (normalized to U6 RNA) were tested in aortic samples of WT rats and SHRs by RT-qPCR assay. **P < 0.01 vs. WT, ***P < 0.001 vs. WT.

remains controversial what role miR-29b plays in the progression of hypertension.

In this study, a model of spontaneously hypertensive rats (SHRs) was used to investigate the role of miR-29b in regulating hypertension and vascular endothelial dysfunction in SHRs. Our studies demonstrated that miR-29b was increased in the aorta in SHRs, and induced hypertension and vascular endothelial dysfunction by targeting CTRP6. Our findings verified that miR-29b triggered Ang II-induced hypertension and vascular endothelial dysfunction is mediated in part by CTRP6/ERK/PPARγ axis. These results suggest that miR-29b plays an important role in the development of hypertension and vascular endothelial dysfunction and may act as a potential target for new antihypertensive drugs.

2. Materials and methods

2.1. Spontaneously hypertensive rats (SHRs)

Male wild type (WT) and spontaneously hypertensive Wistar rats aged 12 weeks were purchased from the Experimental Animal Center of Shandong University (Qingdao City, China). The rats were monitored in their home cage in a stress-free environment where they were provided food and water ad libitum in a humidity-(50% ± 5%) and temperature-controlled (22 ± 2 °C) room under a 12-h light/dark cycle. All animal procedures described herein were approved by the Animal Ethics Committee of Huaihe Hospital of Henan University (Kaifeng, China).

2.2. Blood pressure measurement

Forty male WT Wistar rats (weighting 285 ± 21 g) and sixty male SHRs (weighting 236 ± 20 g), were enrolled in this study. The rats

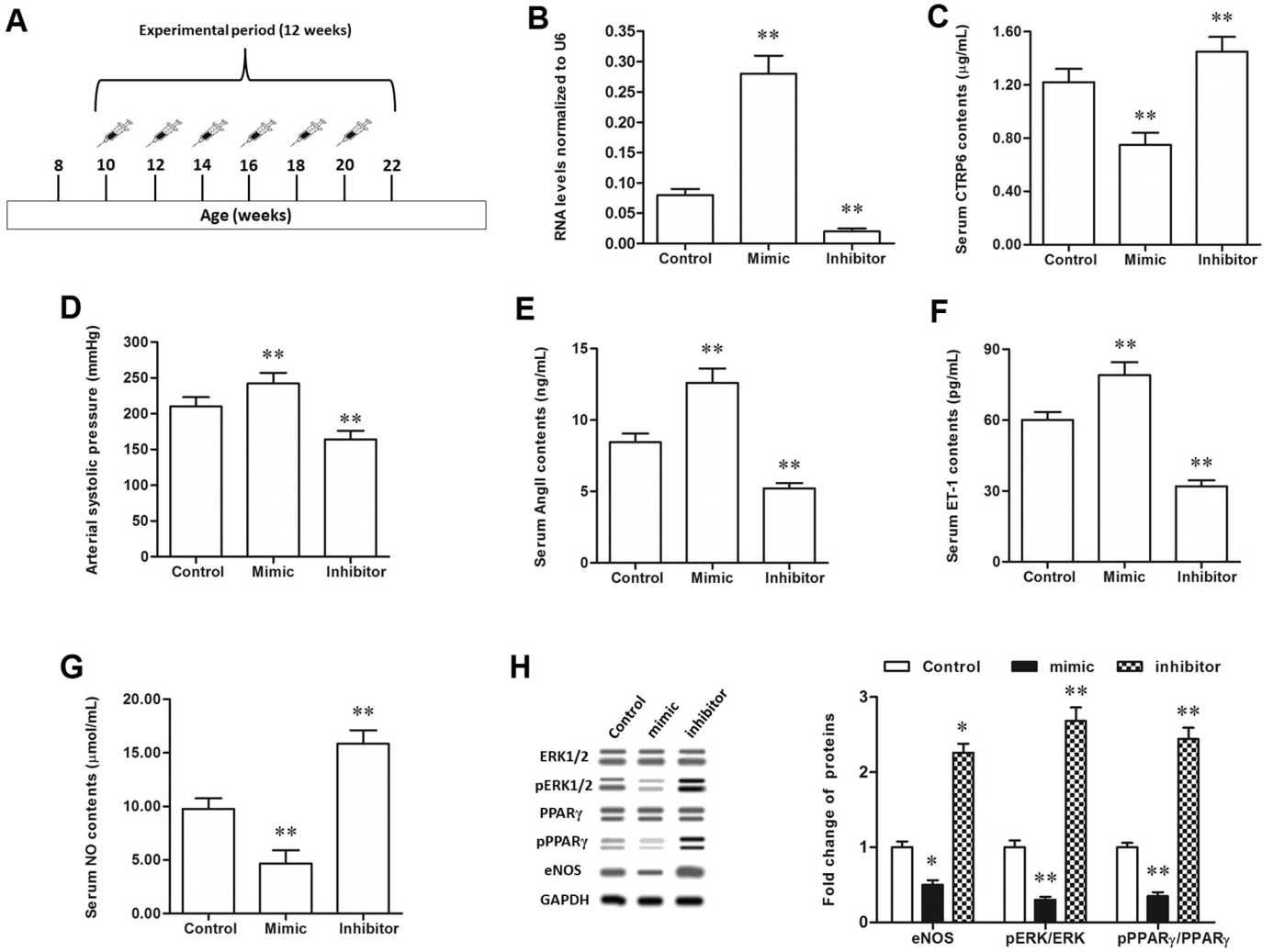

Fig. 2. Effects of miR-29b mimic or inhibitor on hypertension and endothelial function and ERK/PPARγ pathway in SHRs. 45 Rats were divided into three groups at random. Intraventricular injection was used to deliver the miR-29b mimic or miR-29b inhibitor (4 mg/kg) once two weeks from 10th week. Rats in the control group were injected with saline of the same volume with the same method. (A) Experimental process diagram of the administration in vivo. (B) The level of miR-29b was detected in SHRs by RT-qPCR assay after treated with miR-29b mimic or inhibitor. (C, E–G) Serum concentrations of CTRP6, Ang II, ET-1 and NO were tested in SHRs by using ELISA kits. (D) Arterial systolic pressures were detected after treated with miR-29b mimic or inhibitor. (H) Quantitative analysis of eNOS, p-ERK and p-PPARγ expression in the aorta of SHRs by Western blotting. *P < 0.05 vs. Control, **P < 0.01 vs. Control.

were adapted to the conditions for at least two-weeks before experiments. Then, blood pressure was monitored in conscious rats by the noninvasive tail-cuff method using an animal sphygmomanometer (BP98AWU, Softron, Tokyo, Japan). Arterial systolic pressure was measured with multiple readings, 12 stable measurements were obtained and averaged for each rat.

2.3. Serum analysis and aorta sampling

In brief, the rats were anesthetized using 5% chloral hydrate (6 mL/kg, i.p.), and then blood samples were collected via abdominal aorta puncture and serum was isolated. The serum samples were centrifuged at 2000 rpm for 20 min and required for the CTRP6, Ang II, NO and ET-1 levels with ELISA kits, including CTRP6 ELISA kit (Jianglai Biotech, Shanghai, China), ANG II (Angiotensin II) BioAssay ELISA Kit (396365, USBiological, Swampscott, MA), NO ELISA kit (FA01893B, Yantuo Biotech, Shanghai, China) and ET-1 ELISA Kit (GD-QX3292, Guduo Biotech, Shanghai, China). For obtention of the aorta, rats were sacrificed by cervical dislocation, and the thoracic cavity was cut layer by layer, and the aorta was separated. The adipose tissue and fibrous tissue of adventitia were removed as far as possible with ophthalmic scissors and forceps. All the measurements using the commercial kits were carried out in accordance with the instructions from manufacturers. All tests were carried out at least in triplicate.

2.4. Groups and administration

The rats were randomly divided into several groups: WT/control group (injected with PBS), SHRs group, mimic-NC group (injected with negative control mimic), mimic group (injected with miR-29b mimic), anta-NC group (injected with negative control inhibitor), anta-29b inhibitor group (injected with miR-29b inhibitor). Intraventricular injection was used to deliver the miR-29b mimic or miR-29b inhibitor (4 mg/kg) once two weeks from 10th week. The mimics or inhibitors were designed and synthesized by Ribobio Company (Guangzhou, China).

2.5. Aortic endothelial cell isolation and treatment

Aortic endothelial cells were isolated from SHRs in accordance with a previously described method [13,14]. The cells were cultured in DMEM/F12 with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2

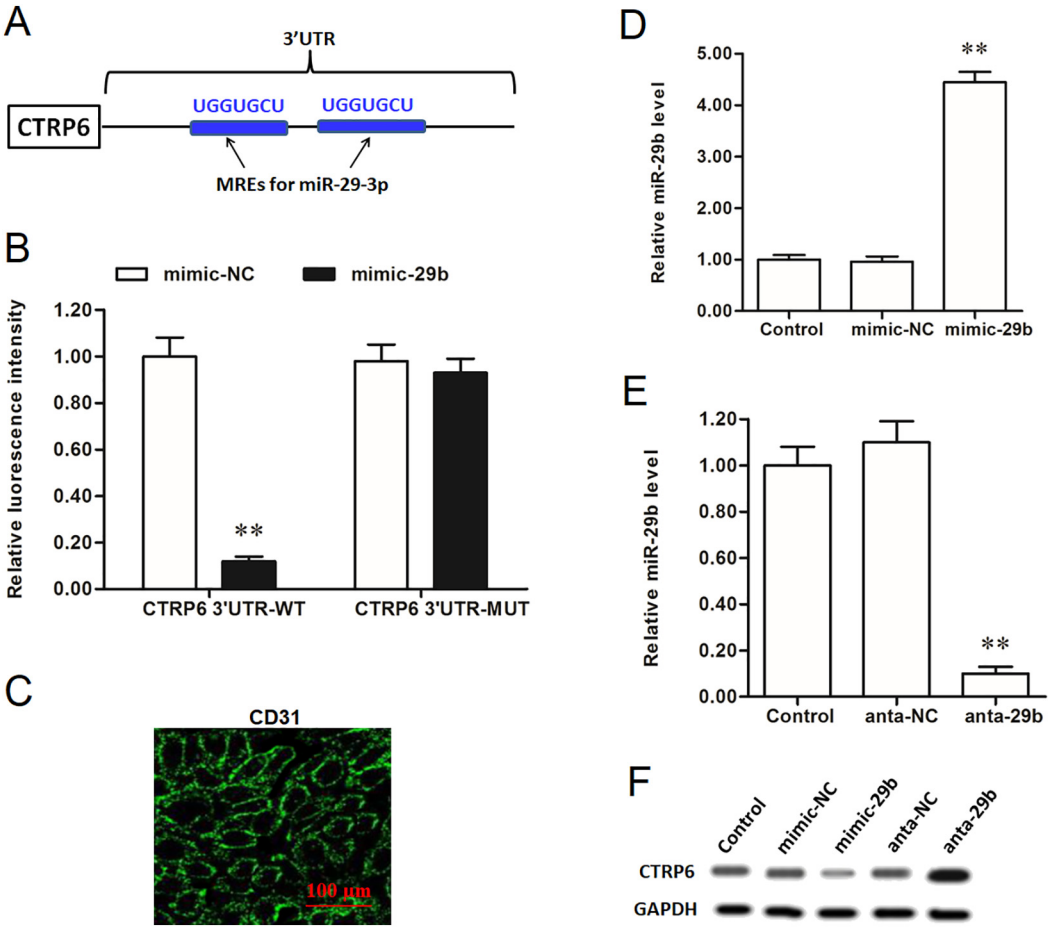

Fig. 3. MiR-29b directly targets and suppresses expression of CTRP6. (A) Prediction result of miR-29b targeting CTRP6 3′-UTR. (B) MiR-29b binding to the CTRP6 3′-UTR was validated in HEK293 cells by using a dual-luciferase reporter assay. Then, primary aortic endothelial cells were isolated and cultured in vitro, (C) which were identified by using immunofluorescence assay for the endothelial surface marker protein CD31 (×200). 40 nM of miR-29b mimic, miR-29b inhibitor and responding controls were respectively transfected into the cells. (D) After 72 h, levels of miR-29b were determined in aortic endothelial cells after treated with miR-29b mimic by RT-qPCR assay. (E) Levels of miR-29b were determined in aortic endothelial cells after treated with miR-29b inhibitor by RT-qPCR assay. (F) CTRP6 protein level was determined with Western blotting. n = 5 for each group. **P < 0.01 vs. Control.

atmosphere in an incubator. Aortic endothelial cells were identified by CD31/vWF immunofluorescence. The cell culture purity was detected to be 90% for subsequent experiments. When the cell density reached 80%, 40 nM miR-29b mimic, 40 nM miR-29b inhibitor, 10 μg/mL mouse CTRP6 recombinant protein (Sino Biological, Beijing, China), or 10 μg/mL mouse CTRP6 recombinant protein plus 40 nM miR-29b mimic, was respectively added into the medium, then cells cultured for another 48 h.

2.6. Western blotting analysis

Tissue total protein was extracted with a Tissue Protein Extraction Kit (BB-3122-2, BestBio, Beijing, China). Cell total protein was extracted with Cell Protein Extraction Kit (BB-3101, BestBio). The Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA) was used to quantify protein concentrations. Equal amounts of protein were electrophoresed onto 12% SDS-PAGE at 100 V for 3 h and transferred to PVDF membranes at 200 mA for 2 h. The membranes were blocked in 5% non-fat milk in TBS-T at 4 °C overnight, then incubated with various primary antibodies, including anti-CTRP6 (1: 300 dilutions, Abcam, USA), anti-ERK (1: 200, Abcam), anti-p-ERK (1: 200, Abcam), anti-PPARg antibody (1: 200, Abcam), anti-p-PPARγ antibody (1: 200, Abcam), anti-eNOS (1: 300, Abcam) and anti-GAPDH (1: 800, Abcam). The membranes were then incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1: 2000, Abcam) in TBS-T at room temperature for 1 h. The blots were visualized using a chemiluminescence system (ECL), and then exposed in a ChemiDoc XRS imaging system and analyzed with Quantity One software (Bio-Rad).

2.7. Dual-luciferase reporter assay

The 3′-untranslated-region (3′-UTR) of CTRP6 mRNA was amplified by PCR using a pair of specific primers and was then cloned into a pMiR-Report vector system (Ambion Inc., Austin, Texas, USA). The mutated 3′-UTR, which is lack of the two binding sites, was amplified by nested PCR using 2 pairs of specific primers. HEK293 cells were transfected with the reporter vectors together with the mimic-NC or mimic-29b. After 48 h, the results were expressed as relative luciferase activity (Firefly/Renilla) and measured using a dual luciferase reporter assay system (Promega, WI, USA). All experiments were repeated three times in triplicate.

2.8. RT-qPCR assay

Tissue and cell total RNA samples were extracted using Trizol (Invitrogen). Next, 2 μg of RNA was reversely transcribed with the RevertAid First Strand cDNA synthesis kit (Fermentas, Canada). For miR-29b detection, the TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) was used for RNA reverse transcription. The expression of miR-29b was determined using the TaqMan

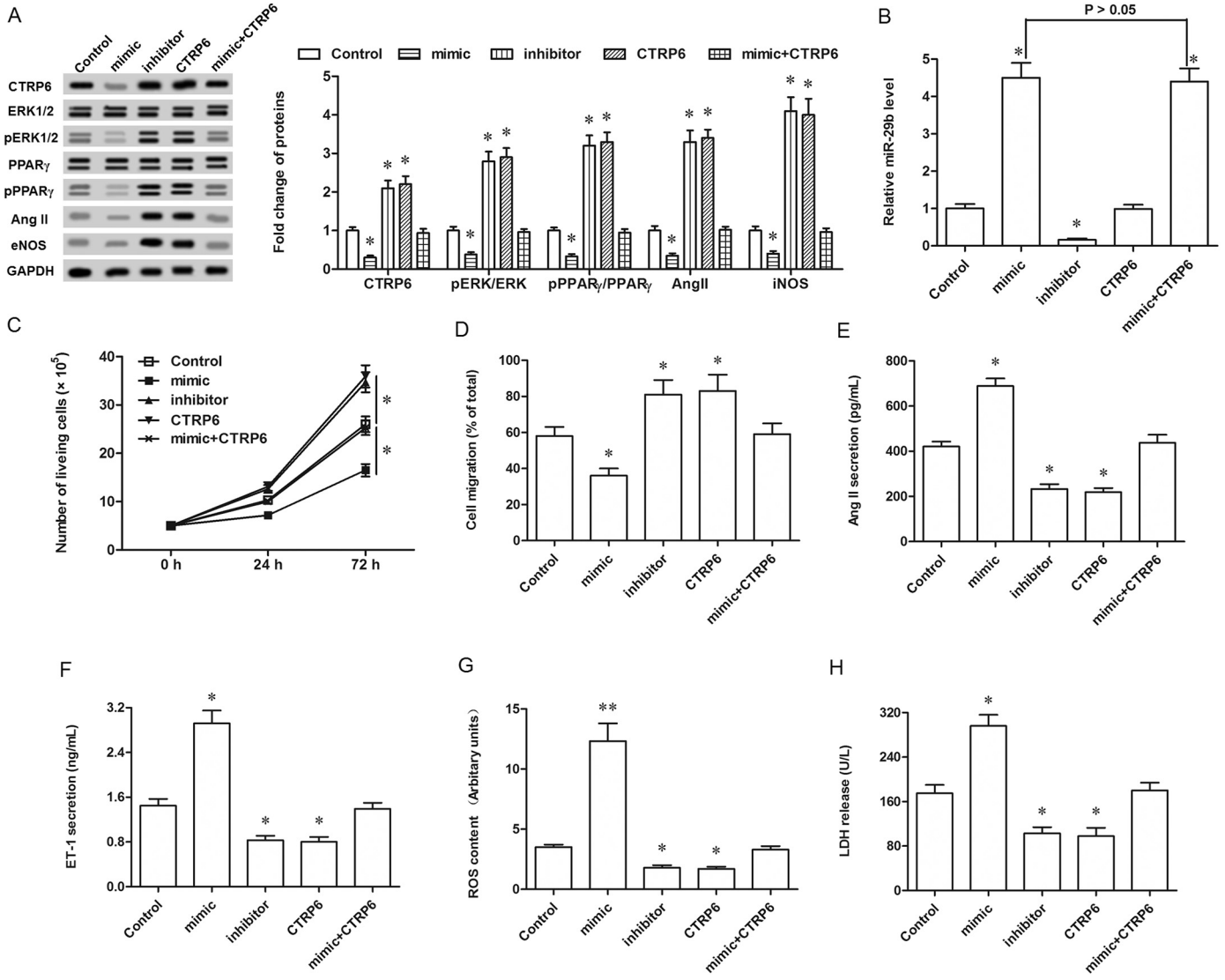

Fig. 4. Inhibition of miR-29b could alleviate Ang II-induced endothelial dysfunction via upregulation of the CTRP6/ERK/PPARγ pathway. Aortic endothelial cells were isolated and cultured in vitro. 40 nM miR-29b mimic, 40 nM miR-29b inhibitor, 10 μg/mL mouse CTRP6 recombinant protein (Sino Biological, Beijing, China), or 10 μg/mL mouse CTRP6 recombinant protein plus 40 nM miR-29b mimic, was respectively added into the medium. When incubated for 72 h, (A) the expression of CTRP6, ERK, p-ERK, PPARγ, p-PPARγ, Ang II and eNOS were detected with Western blotting. (B) MiR-29b expression was detected with RT-qPCR analysis. (C) cell proliferation was measured by an automatic counter. (D) Migrated cell number was measured by the Transwell migration assay. (E) and (F) The secretion of Ang II and ET-1 in the supernatant was detected with Ang II and ET-1 ELISA kits. (G) The change of ROS content was determined by ROS indicator $DCFH_2$-DA. (H) The change of LDH release was determined by LDH assay kit. n = 5 for each group. *P < 0.05 vs. Control, **P < 0.01 vs. Control.

2× Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95 °C for 5 min followed by 40 cycles of 95 °C for 15 s, then 58 °C for 30 s and 72 °C for 20 s. The reactions were all performed three times. The data were analyzed with quantitative Rotor-Gene software. The relative expression levels of miR-29b tested were calculated using the $2^{-\Delta\Delta CT}$ method and normalized to GAPDH.

2.9. Migration assay

Migration assays were performed by using 8-μm pore size filters within Transwell cell culture chambers with polycarbonate filters (Corning Costar, Cambridge, MA, USA). Primary aortic endothelial cells (5×10^5) were pretreated with miR-29b mimic or miR-29b inhibitor, and then seeded into the upper of the Transwell chambers. As a chemoattractant, MCP-1 (100 ng/mL; R&D Systems) was present in the lower wells. After incubation for 48 h at 37 °C in 5% CO_2, cells in the lower chambers that passed through the filter were counted under a Carl Zeiss Primo Vert microscope (Carl Zeiss, Toronto, ON, Canada).

2.10. Cell proliferation

The cell growth was measured by automatic counter. In brief, cells were seeded onto 6-well plates and the density was 5×10^5 cells/well. The cell numbers were determined using the ADAM-MC automatic cell counter (NanoEn Tek, Seoul, Republic of Korea) after seven days. All experiments were repeated three times.

2.11. ROS content analysis

The primary aortic endothelial cells were transfected with the miR-29b mimic or inhibitor and incubated at 37 °C for 48 h. The cells were collected, washed with PBS, and performed using a fluorescence ROS indicator DCFH2-DA (2.5 μM), as previously reported (Pasqualini et al. 2011). ROS contents were detected by 2.5 μM DCFH2-DA for 1 min, followed by a wash. Imaging was carried out using the fluorescence

microscope (AxioImager).

2.12. LDH release assay

LDH release was quantified using a colorimetric LDH cytotoxicity detection kit (Clontech, Mountain View, CA, USA), according to the manufacturer's manual. LDH activity was measured via a colorimetric assay using an absorption wavelength of 492 nm in a spectrophotometer (Bio-Rad Laboratories, Hercules, CA). The background absorbance was subtracted and the percentage of LDH release was calculated based on the LDH standard curve.

2.13. Statistical analyses

All statistical values are represented as the mean ± SD. Statistical significance was determined using Student's *t*-test when there were two experimental groups. When more than two groups were compared, statistical evaluation of the data was performed by one-way analysis of variance (ANOVA). Statistically significant was set at $P < 0.05$.

3. Results

3.1. CTRP6 is downregulated whereas miR-29b is upregulated in SHRs

To determine the potential roles of miR-29b and CTRP6 in hypertension, CTRP6 content was assessed in the serum, and levels of three miR-29 members were assessed in the aorta of WT and SHRs rats. Before that, the arterial systolic pressures, several specific markers of hypertension and vascular endothelial dysfunction in rats were detected. In the SHRs group, apparently, rats had higher arterial systolic pressures than rats in the WT group (Fig. 1A). Moreover, compared with WT group, plasmatic concentrations of Ang II and ET-1 were significantly increased, while the NO and CTRP6 levels decreased in SHRs serum (Fig. 1B–E). It has also recently been shown that miR-29 is closely associated with hypertension. The level of miR-29b was aberrantly up-regulated in SHRs group, but levels of miR-29a and miR-29c displayed no difference between WT rats and SHRs, suggesting that a potential function of miR-29b in hypertension (Fig. 1F). These results exhibited that SHRs showed decreased serum CTRP6 content and increased aortic miR-29b level, accompanied by a distinct increase in both arterial systolic pressures and biomarkers of vascular endothelial dysfunction.

3.2. Inhibition of miR-29b reduced Ang II secretion and alleviated hypertension and endothelial dysfunction in SHRs by activating ERK/PPARγ pathway

To further confirm the effect of miR-29b in hypertension and endothelial function, miR-29b mimic or inhibitor were intraventricularly injected into the SHRs (Fig. 2A). After a 12-weeks injection period, experiments results indicated that miR-29b level was remarkably up-regulated, whereas CTRP6 was significantly decreased after miR-29b overexpression (Fig. 2B–C). Meanwhile, miR-29b inhibitor treatment resulted in a prominent decrease in the miR-29b level, and a notable increase in CTRP6 (Fig. 2B–C). In addition, compared with the control, arterial systolic pressure and serum concentrations of Ang II and ET-1 were lowered by miR-29b inhibitor (Fig. 2D–F). Serum NO content was enhanced by miR-29b inhibition (Fig. 2G). To determine whether reduced miR-29b alleviated hypertension and endothelial dysfunction via ERK/PPARγ signaling, we measured ERK/PPARγ activation by Western blotting (Fig. 2H). We observed that inhibition of miR-29b enhanced the expression of p-ERK, p-PPARγ and eNOS in the aorta of SHRs. However, the effects of miR-29b inhibitor on hypertension, endothelial dysfunction and ERK/PPARγ pathway were opposite to those of miR-29b mimic. These results suggest that repressed miR-29b reduced Ang II expression and alleviated hypertension and endothelial dysfunction in SHRs partially through activating the ERK/PPARγ pathway.

3.3. CTRP6 is a direct target of miR-29b

To identify the underlying mechanism of miR-29b in regulating hypertension and endothelial dysfunction, CTRP6, the putative target gene of miR-29b, was screened by using the online bioinformatics software TargetScan. CTRP6 has been suggested to play a favorable role in the Ang II-induced hypertension and vascular endothelial dysfunction. As shown in Fig. 3A, the 3′-UTR of CTRP6 contained two potential miR-29b binding sites. Luciferase activity assay showed that miR-29b mimic significantly reduced luciferase activity of WT reporter vector, indicating miR-29b could suppress the translation of CTRP6, but it had no effect on the mutated reporter vector (Fig. 3B). Then, primary aortic endothelial cells were isolated and cultured in vitro, which were identified by using immunofluorescence assay for the endothelial surface marker protein CD31 (Fig. 3C). The miR-29b mimic, miR-29b inhibitor and responding controls were respectively transfected into the cells. The level of miR-29b was distinctly up-regulated by miR-29b mimic transfection and downregulated by miR-29b inhibitor transfection (Fig. 3D and E). Furthermore, the effect of miR-29b inhibitor on CTRP6 expression was also validated in primary aortic endothelial cells by Western blotting, the results of which indicated that protein level of CTRP6 was greatly increased by inhibition of miR-29b (Fig. 3F).

3.4. Inhibition of miR-29b improved CTRP6/ERK/PPARγ-mediated endothelial function, and CTRP6 recombinant protein could antagonize the suppressive effect of miR-29b on activation of the ERK/PPARγ axis and function of the endothelial cells

Finally, to verify the regulation of miR-29b on Ang II secretion and vascular endothelial dysfunction is associated with CTRP6-mediated activation of the ERK/PPARγ axis. The expression of miR-29b was manipulated in mouse primary aortic endothelial cells. Meanwhile, mouse CTRP6 recombinant protein was applied to treat the primary aortic endothelial cells alone or together with miR-29b mimic. The results showed that the CTRP6 recombinant protein incubation increased the level of CTRP6 protein in the endothelial cells (Fig. 4A) but had no effect the level of miR-29b (Fig. 4B). The CTRP6 recombinant protein, displaying a similar effect to miR-29b inhibition, could activate the ERK/PPARγ axis and reduce production of Ang II and eNOS (Fig. 4A), improve the proliferation and migration of endothelial cells (Fig. 4C and D), suppress the secretion of Ang II and ET-1 (Fig. 4E and F), and reduce ROS accumulation and LDH release (Fig. 4G and H). Moreover, the CTRP6 recombinant protein could antagonize the suppressive effect of miR-29b on activation of the ERK/PPARγ axis (Fig. 4A) and function of the endothelial cells (Fig. 4C–H). These results demonstrated that inhibition of miR-29b could alleviate Ang II-induced endothelial dysfunction via upregulation of the CTRP6/ERK/PPARγ pathway.

4. Discussion

C1q/tumor necrosis factor-regulated protein 6 (CTRP6), a subtype of the CTRP adipocytokine family (CTRP1-15), has been implicated in regulating lipid and glucose metabolism, and inflammation [1]. Knockdown of CTRP6 can inhibit adipogenesis of 3T3-L1 adipocytes via lipogenic marker genes and ERK1/2 pathway [15], which indicated that CTRP6 increased the expression of IL-10 in macrophages through ERK1/2 activation, and CTRP6 may provide a new targeting point for molecular-based therapy for inflammatory diseases [16]. A recent study showed that CTRP6 was able to prevent the Ang II-induced hypertension and vascular dysfunction through proliferator-activated receptor γ (PPARγ) activation [1].

In this study, we found that miR-29b was significantly up-regulated in the aortic samples of SHRs and exerted an adverse effect on

hypertension and endothelial dysfunction, manifested by increased arterial systolic pressure, enhanced serum Ang II and ET-1 level, and decreased NO level and CTRP6 expression. Endothelial dysfunction, at the cellular level, are manifested by diminished proliferation and migration, and increased ROS and LDH production. Inhibition of miR-29b, in our current study, displayed an effect on improvement of proliferation and migration, and suppression on production of ROS and LDH. To further confirm the role of miR-29b in hypertension and endothelial, miR-29b mimic or inhibitor were intraventricularly injected into the SHRs. Here, there was evidence that repressed miR-29b reduced Ang II secretion and alleviated hypertension and endothelial dysfunction in SHRs by activating ERK/PPARγ pathway. Overexpression of miR-29b aggravated the hypertension and endothelial dysfunction in the aortic samples of SHRs. In addition, these conclusions have also been verified in the aortic endothelial cells isolated from SHRs. The surprise result contrasted sharply with earlier studies showing that up-regulated miR-29b has a protective effect on ischemic stroke, neuronal cell death and ataxia, even on Ang II-induced cardiac fibrosis [9,10,17]. However, in consistent with our present study, a couple of recent studies revealed that miR-29 family were significantly upregulated in patients with hypertension and positively correlated with their blood pressure. One possible explanation of this contradiction is that it is due to a context-dependent manner for miR-29b in complex pathological microenvironments [18]. In this study, the research focused on the changes of miR-29b in cardiovascular system rather than the peripheral vascular system. Therefore, consequently diverse impacts on their target genes and resultant diverse phenotypic manifestations are conceivable.

CTRP6 is a novel cytokine that may play a crucial role in compensating for the loss of adiponectin under certain conditions, anti-inflammatory cytokine production and hepatocellular carcinoma [14,19,20]. Previous research has shown that the CTRP6 protects rat from Ang II-induced arterial hypertension and vascular endothelial dysfunction [1]. Here, we reconfirmed that CTRP6 was down-regulated in the serum of SHRs and identified as a direct target gene of miR-29b. These data suggest that reduction of CTRP6 could be one of the contributing factors leading to hypertension and vascular endothelial dysfunction in SHRs. Loss of miR-29b directly enhanced CTRP6 expression. Down-regulation of miR29b significantly increased the number of live cells and cells migration in aortic endothelial cells of SHRs, while decreased the ROS content and LDH release. Our results presented herein reinforce the concept that miR-29b antagonism attenuates Ang II-induced hypertension and vascular endothelial dysfunction by targeting CTRP6.

PPARγ, as a member of the nuclear receptor superfamily of ligand activated transcription factors, has emerged at the crossroads of metabolic and cardiovascular diseases [21]. It has been reported that PPARγ has certain vascular effects which are of great importance for cerebral circulation and provide genetic evidence that activation of PPARγ function specifically in protecting blood vessels [22]. However, ERK promotes PPARγ translocation from nucleus to cytoplasm which leads to the phosphorylation of PPARγ [23,24]. Over-activated of ERK is a primary cause for impaired transcriptional PPARγ in type 2 diabetes patients' adipocytes [25,26]. In our present study, we provided strong evidence that miR-29b inhibitor remarkably promoted the activation of the CTRP6/ERK/PPARγ axis in SHRs. In conclusion, findings suggested that inhibitory effects of miR-29b on Ang II-induced hypertension and vascular endothelia dysfunction are dependent on the CTRP6/ERK/PPARγ axis pathway.

Collectively, the present study provides insight into repressed miR-29b in Ang II-induced hypertension and vascular endothelial dysfunction in rat through a mechanism involving activation of CTRP6/ERK/PPARγ axis pathway. Given the alleviating action of miR-29b on the development of hypertension, it holds-promise as a new target of gene therapy for patients with hypertension.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

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