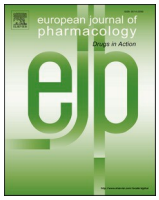
European Journal of Pharmacology 962 (2024) 176252

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



The role of angiotensin II activation of yes-associated protein/PDZ-binding motif signaling in hypertensive cardiac and vascular remodeling

Qian Xu [a,b,1], Kunping Zhuo [b,1], Xiaotian Zhang [b], Yanru Zhen [b], Limin Liu [c], Lu Zhang [a,b], Yufan Gu [b], Hui Jia [d], Qing Chen [e], Meixi Liu [f], Jiawei Dong [f], Ming-Sheng Zhou [a,b,*]

[a] Science and Experiment Research Center, Shenyang Medical College, Shenyang, China
[b] Department of Physiology, Shenyang Medical College, Shenyang, China
[c] Department of Vasculocardiology, The Second Hospital of Shenyang Medical College, Shenyang, China
[d] Department of Traditional Chinese Medicine, Shenyang Medical College, Shenyang, China
[e] Department of Pharmacy, Shenyang Medical College, Shenyang, 110034, China
[f] Department of Clinical Medicine, School of Basic Medicine, Shenyang Medical College, Shenyang, China

ARTICLE INFO

Original content: original data and Supplementary data (Original data)

Keywords:
Ang II
Cardiovascular diseases
YAP/TAZ
Hypertension
Vascular remodeling

ABSTRACT

Vascular remodeling is the pathogenic basis of hypertension and end organ injury, and the proliferation of vascular smooth muscle cells (VSMCs) is central to vascular remodeling. Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are key effectors of the Hippo pathway and crucial for controlling cell proliferation, apoptosis and differentiation. The present study investigated the role of YAP/TAZ in cardiac and vascular remodeling of angiotensin II-induced hypertension. Ang II induced YAP/TAZ activation in the heart and aorta, which was prevented by YAP/TAZ inhibitor verteporfin. Treatment with verteporfin significantly reduced Ang II-induced cardiac and vascular hypertrophy with a mild reduction in systolic blood pressure (SBP), verteporfin attenuated Ang II-induced cardiac and aortic fibrosis with the inhibition of transform growth factor (TGF)β/Smad2/3 fibrotic signaling and extracellular matrix collagen I deposition. Ang II induced Rho A, extracellular signal-regulated kinase 1/2 (ERK1/2) and YAP/TAZ activation in VSMCs, either Rho kinase inhibitor fasudil or ERK inhibitor PD98059 suppressed Ang II-induced YAP/TAZ activation, cell proliferation and fibrosis of VSMCs. Verteporfin also inhibited Ang II-induced VSMC proliferation and fibrotic TGFβ1/Smad2/3 pathway. These results demonstrate that Ang II activates YAP/TAZ via Rho kinase/ERK1/2 pathway in VSMCs, which may contribute to cardiac and vascular remodeling in hypertension. Our results suggest that YAP/TAZ plays a critical role in the pathogenesis of hypertension and end organ damage, and targeting the YAP/TAZ pathway may be a new strategy for the prevention and treatment of hypertension and cardiovascular diseases.

1. Introduction

Hypertension affects over one billion people worldwide and is a major risk factor for the development of cardiovascular diseases, sub-optimal control of blood pressure is the leading attributable risk for cardiovascular mortality (Slivnick and Lampert, 2019). Long-term high blood pressure can alter the structure and function of the heart and blood vessels and promote premature aging phenotype of blood vessels, characterized with vascular remodeling, inflammation and endothelial dysfunction (Gallo et al., 2021; Yildiz et al., 2020). Vascular remodeling is an active process that involves the proliferation, apoptosis, fibrosis and hypercontractility of vascular smooth muscle cells (VSMCs), as well as the imbalance between extracellular matrix (ECM) accumulation and degradation, leading to an increase in vascular stiffness, wall thickness, and reduced distensibility (Humphrey, 2021; Liu et al., 2022). Vascular fibrosis and remodeling are main pathological processes that promote hypertension related cardiovascular diseases (Cai et al., 2021).

The cellular mechanisms and intracellular signaling events of cardiovascular remodeling in hypertension are complex. Many factors have been implicated in the pathophysiological process of cardiovascular remodeling in hypertension, with the activation of renin-angiotensin aldosterone system (RAAS) being one of the most significance

* Corresponding author. Science and Research Center, Shenyang Medical College, Huanghe N St. 146, Shenyang, 110034, China.
E-mail address: Zhoums1963@163.com (M.-S. Zhou).
[1] Equally contributed to this work.

https://doi.org/10.1016/j.ejphar.2023.176252
Received 2 July 2023; Received in revised form 1 December 2023; Accepted 1 December 2023
Available online 5 December 2023

(Martyniak and Tomasik, 2022). Ang II is the effector of RAAS, mainly exerts its biological effects by binding to the angiotensin II type I receptor (AT1R) (Ashour, 2022). At the cellular level, Ang II stimulates AT1R to activate multiple signaling pathways, such as mitogen-activated protein kinases (MAPKs), Rho kinase and nuclear factor (NF)κB, which may increase the proliferation, fibrosis, oxidative stress of cardiomyocyte and VSMCs, ECM accumulation and endothelial dysfunction, thereby promoting cardiovascular remodeling (Bregeon et al., 2009; Kawai et al., 2017; Zhao et al., 2019).

There are increasing evidence showing that the Hippo/Yes-associated protein (YAP) pathway plays a role in cardiovascular remodeling and related CVDs (Li and Huang, 2023; Zheng et al., 2022). The Hippo pathway controls organ size by regulating survival, proliferation and apoptosis, and YAP and its associated PDZ-binding motif (TAZ) are key downstream effectors of the Hippo pathway (Moya and Halder, 2019). YAP is an important molecule to sense biochemical force and hemodynamic stress (Wang et al., 2016). Altered ECM stiffness and thickness in cardiac and vascular wall and disturbed blood flow associated with hypertension, may be a potent activator of YAP/TAZ (Dupont et al., 2011; Liu et al., 2019). In turn, the activation of YAP/TAZ may further promote cardiovascular remodeling by altering ECM production/degradation, VSMC growth and death, and vascular inflammation (Yamashiro et al., 2020; Zheng et al., 2022). In addition, there is a complex interaction between YAP/TAZ and Ang II singling in cardiovascular system (Lin et al., 2018)

We have recently shown that Ang II activates YAP/TAZ signaling to induce endothelial dysfunction, vascular inflammation and renal injury in hypertensive mice (Xu et al., 2021; Zhang et al., 2021). In this study, we investigated the role and potential signaling mechanisms of YAP/-TAZ activation in Ang II-induced cardiovascular remodeling in hypertensive mice.

2. Materials and methods

2.1. Animal and experimental protocols

Six-week-old male C57BL/6 mice were purchased from the Charles River Animal Laboratory in Beijing (China). These mice were housed in the animal facility of Shenyang Medical College and free access to tap water and mice diet. All animal experimental protocols complied with the international standards stated in the guide for the Care and Use of Laboratory Animals. All animal protocols were approved by the Institutional Animal Care and Use Committee of Shenyang Medical College (SYYXY2021110101). After adapting to the environment for 2 weeks, mice were randomly divided into three groups and treated for 3 weeks: (1) control group (Ctr): mice underwent a sham surgery with the implantation of an empty osmotic mini-pump (n = 6); (2) Ang II group (Ang II): mice were implanted with an osmotic mini-pump (Alzet model 1002D, DURECT Inco., Cupertino, CA, United States) and infused with Ang II (1.1 mg/kg/day, Sigma-Aldrich, St. Louis, MO, n = 6); (3)Ang II infusion plus verteporfin treatment group (Ang II/Ve): mice were implanted with an osmotic mini-pump and infused with Ang II plus verteporfin (Houston, TX) treatment (n = 6). We have previously shown that Ang II at this pressor dose can induce hypertension and cardiovascular and renal injury in mice (Zhang et al., 2021). The mice in Ang II/Ve group were intraperitoneally injected with 60 mg/kg of verteporfin every other day. Verteporfin was dissolved in 10% DMSO saline solution, to eliminate potential side effects of DMSO solvent, the mice in control and Ang II group were intraperitoneally given with same amount of the solvent every other day. Verteporfin is an inhibitor of YAP, which inhibits YAP activation by disrupting the YAP-TEAD interactions and decreases the protein expression of YAP (Szeto et al., 2016; Wei et al., 2017). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by tail-cuff method (Softron Biotechnology Co., Ltd., Beijing, China) at quiet and dark room in the conscious mice as previously described (Huang et al., 2018). The mice were trained daily for 5 consecutive days before the experiments started. SBP and DBP were measured at baseline (before mini-pump implantation) and every week thereafter. Record at least five successive readings and average them as a single value for each mouse. Mice were euthanized by an overdose of anesthetic (5% chloral hydrate 0.4 mL/100 g body weight, I.P.). The aorta and hearts were harvested and snap frozen in liquid nitrogen.

2.2. Histological examination

The thoracic aorta (just below the highest point of the aortic arch) and one piece of heart tissue were fixed in 4% paraformaldehyde in phosphate-buffered saline. The tissue samples were embedded in paraffin and cut into 4 μm-thick sections, and stained with hematoxylin eosin (Sigma Aldrich, St. Louis, MO) or wheat germ agglutinin (WGA ，Beijing Solarbio Science & Technology Co.,Ltd.) . Four digital images per sample were acquired using a Leica DM4B fluorescence microscope (Leica Microsystems Inc., Mannheim, Germany) and analyzed with ImageJ 1.48V software, average aortic wall thickness and myocardial cell area were measured. Masson-trichrome staining was used to evaluate vascular fibrosis. The percentage of positive stained area was assessed with ImageJ software as a semi-quantitative analysis of collagen content in vascular tissue. The slide examination, image quantitation and representative photomicrographs were taken in a blinded manner, and the reviewer was unaware of the experimental groups.

2.3. VSMC culture

The VSMC line A7R5 derived from the thoracic aorta of rat was obtained from American Type Culture Collection (ATCC, Manassas, VA), and cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, NY) supplemented with 10% fetal bovine serum, 100 U/ml streptomycin and 100 U/ml penicillin in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were used between passage 4 and passage 12 with 60% confluence and seeded in six-well plates (2×10^5 cells/well), and starved in serum-free DMEM medium for 24 h before the experiments were performed. Cells were incubated with Ang II (100 nmol/L) for 6–24 h. In some experiments, cells were preincubated with YAP inhibitor verteporfin (0.5 μmol/L), Rho A inhibitor fasudil (10 μmol/L) or ERK inhibitor PD98059 (20 μmol/L) before Ang II was applied.

2.4. CCK-8 assay

VSMC proliferation was determined using a CCK-8 assay kit (Vazyme, Nanjing, China). Growing cells were diluted to 2×10^4 cells/ml and seeded in 96-well plates, cells were incubated with Ang II (100 nmol/L) or Ang II plus the indicated inhibitor for 24 h, 10 μl CCK-8 solution was added to well followed by the incubation for 2 h at 37 °C. Absorbance at 450 nm was measured with a microplate reader (BGM LABTECH, Germany). Samples were run in triplicate.

2.5. Immunofluorescence

The aortic rings were embedded in paraffin and cut into 4 μm-thick sections. The slides were incubated with sodium citrate buffer (pH 6.0) and placed on a pressure cooker for 10 min for antigen retrieval, the sections were blocked with 5% goat serum in TBS (blocking solution) for 1 h. Then the sections were incubated with primary mouse anti-YAP (1:200 dilution with TBST buffer, SC-101199, Santa Cruz Biotech.) or rabbit anti-collagen I (1:200 dilution with TBST buffer, SC-39357, Santa Cruz Biotech.) antibodies overnight at 4 °C, followed by the incubation with fluorescein (FITC)-conjugated goat anti-mouse secondary antibody for YAP (1:500 dilution with TBST buffer, Beyotime Biotechnology), or fluorescein (Cy3)-conjugated goat anti-rabbit secondary antibody for collagen I (1:300 dilution with TBST buffer, Beyotime Biotechnology.) at 37 °C for 1 h. The nucleus was counter-stained with DAPI. Fluorescence

intensity was visualized and photographed using a Leica DM4B fluorescence microscope (Leica Microsystems Inc., Mannheim, Germany). The relative fluorescence intensity (RFI) of nuclear YAP or collagen I was determined using the ImageJ 1.54 software system and normalized by control group.

2.6. Western blot

The aorta or VSMCs were washed with cold PBS and lysed in RIPA lysis buffer supplemented with 1 mmol/L PMSF, 10 μg/ml aprotinin and 10 μg/ml leupeptin at 4 °C for 60 min. In some experiments, nuclear and cytoplasmic protein fractions were separated and extracted using nucleoprotein extraction kit (BestBio, Shanghai, China) according to the manufacture's protocol. Protein concentration was measured by the Bio-Rad protein assay (Beyotime Biotech., Shanghai, China). Fifty μg of total proteins were separated by 8 or 12% polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane. The membranes were incubated with a blocking solution (5% milk in TBST buffer) at room temperature for 2 h, and then incubated with primary antibodies against YAP (SC-101199, Santa Cruz Biotech.), p-YAP (13008T, Cell Signaling), TAZ (4883S, Cell Signaling), p-TAZ (59971S, Cell Signaling), Rho A (SC-418, Santa Cruz Biotech.), TGFβ (A16640, ABclonal), Smad2/3 (sc-133098, Santa Cruz Biotech.), p-Smad3 (9520, Cell Signaling), collagen I (SC-39357, Santa Cruz Biotech.), HDAC1 (SC-81598, Santa Cruz Biotech.), and GAPDH (60004-1-Ig, Proteintech Group) at 4 °C overnight (1:500 dilution with blocking solution). The membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:5000 dilution using blocking solution) for 2 h at room temperature. The signals of luminal chemiluminescence were detected by an Aplegen Omega Lum G Gel Documentation System (Aplegen Inc., Pleasanton) and quantified by ImageJ. 1.48V software system. HDAC1 was used as a loading control for nuclear lysates, and GAPDH for whole lysates. Data was normalized by control group.

2.7. Statistical analysis

Data was analyzed using GraphPad statistical software package, and expressed as mean ± SE. Statistical significance of difference was determined by one-way or two-way ANOVA with Bonferroni's correction for multiple comparisons. Values were considered significant when $p < 0.05$.

3. Results

3.1. Verteporfin inhibits YAP/TAZ activation in the heart and vessels of Ang II hypertensive mice

YAP and its transcriptional coactivator TAZ are core downstream molecules and cascade effectors of the Hippo signaling pathway. The activation of the Hippo pathway induces YAP/TAZ phosphorylation, resulting in YAP/TAZ inactivation and retention in the cytosolic apartment (Kwon et al., 2022). When the hippo signaling pathway is inactivated, unphosphorylated YAP/TAZ transfers from the cytoplasm into the nucleus and binds to TEAD1-4 to increase the expressions of target genes (Pocaterra et al., 2020). We used immunofluorescence staining to determine nuclear fluorescence intensity of YAP in the aorta and heart. Ang II significantly increased nuclear fluorescence intensity of YAP in the aorta (Fig. 1A&B) and heart (Fig. 1E&F), while treatment with

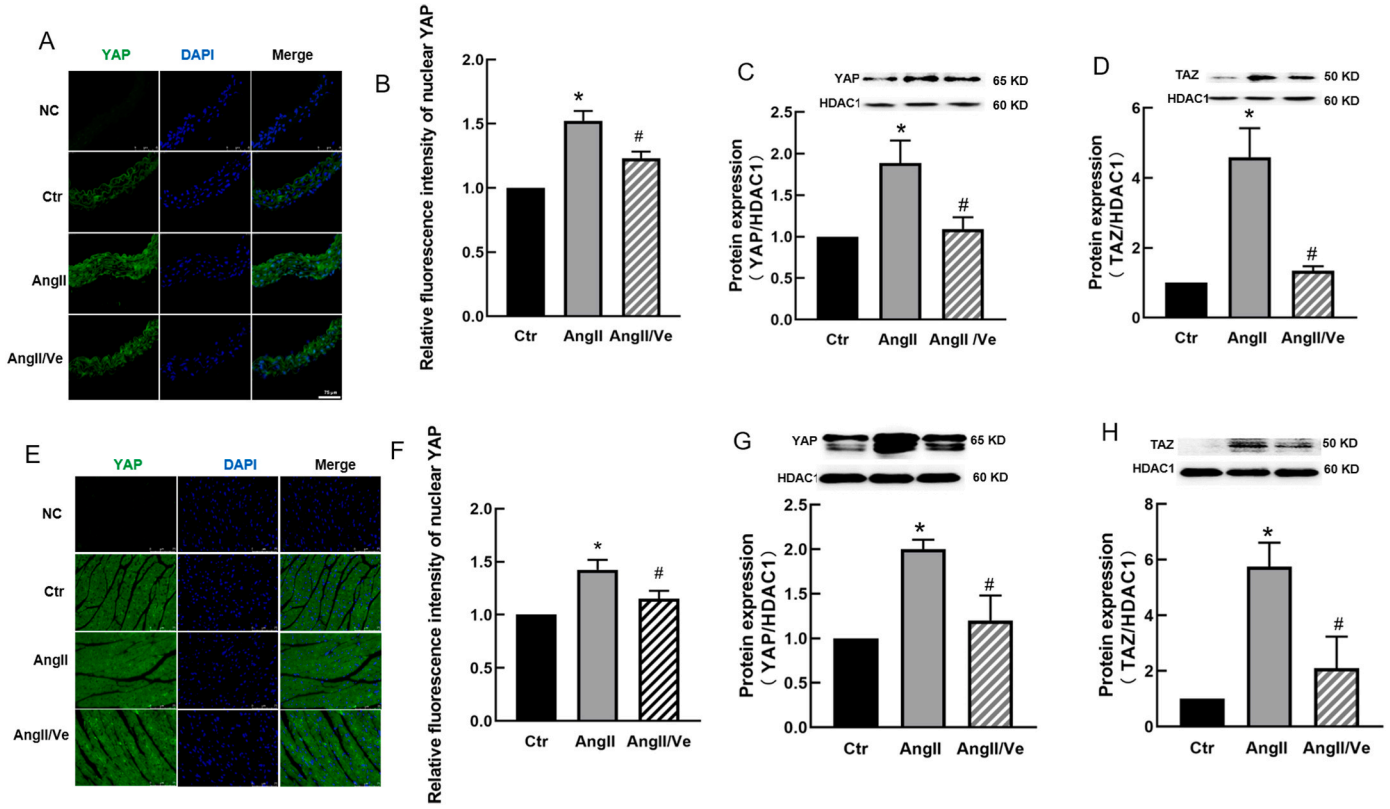


Fig. 1. YAP/TAZ activation in the aorta and heart of Ang II hypertensive mice. Representative images of YAP immunofluorescence in the aorta (A) and heart (E), YAP was immunolabeled with FITC (Green) , cell nucleus stained with DAPI (blue), bar = 75 μm. Quantitative analysis of YAP fluorescence intensity in aortic (B) and myocardial (F) cell nuclei. The protein expression of YAP (C) and TAZ (D) in the nuclear fraction of aorta. The protein expression of YAP (G) and TAZ (H) in the nuclear fraction of heart. Ctr: C57BL/6 mice were implanted with an empty osmotic mini-pump, Ang II: mice were infused with angiotensin II for 3 weeks, Ang II/Ve: mice were infused with Ang II plus verteporfin treatment for 3 weeks. All data were expressed as mean ± SEM. n = 6 *$p < 0.05$ vs. Ctr group; #$p < 0.05$ vs. Ang II group.

verteporfin significantly reduced nuclear fluorescence intensity of YAP. In addition, we separated aortic and cardiac tissues into the nuclear and cytoplasmic fractions, and determined the protein expressions of nuclear YAP/TAZ and cytosolic phosphor-YAP/phosphor-TAZ by Western blot. Ang II increased the protein expression of YAP/TAZ in the nuclear fraction of the aortic (Fig. 1C&D) and cardiac tissues (Fig. 1G&H), and decreased phosphor-YAP/phosphor-TAZ in the cytoplasmic fraction of aortic (Fig. S1 A&B) and cardiac (Fig. S1C&D) tissues. These results indicate that Ang II activates YAP/TAZ signaling pathway in the vascular system and heart, while verteporfin effectively inhibits this pathway.

3.2. *Verteporfin lowers Ang II-induced elevation of blood pressure and cardiac and vascular hypertrophy and fibrosis*

The infusion of Ang II for 3 weeks significantly increased heart weight (4.6 ± 0.2 vs. 3.8 ± 0.1 mg/g body weight in control group, $p < 0.05$, Fig. 2A&B) and cardiomyocyte sectional area (375.0 ± 19.0 vs. 218.7 ± 11.9 μm^2 in control group, $p < 0.05$), as demonstrated by HE (Fig. 2C&D) and WGA staining (Fig. S2). Verteporfin significantly reduced heart weight (3.9 ± 0.1 vs. 4.6 ± 0.2 mg/g body weight in Ang II group, $p < 0.05$, Fig. 2A&B) and cardiomyocyte sectional areas (258 ± 14 vs. 375 ± 19 μm^2 in Ang II group ($p < 0.05$, Fig. 2C&D & Fig. S2). Masson-trichrome staining showed that Ang II significantly increased the positive staining area of collagen in the heart, which was significantly reduced in Ang II/Ve mice (Fig. 2E&F). The infusion of Ang II for 3 weeks significantly increased SBP (165 ± 4 vs. 111 ± 2 mmHg in control group, $p < 0.05$, Fig. 3A) and DBP (102 ± 3 vs. 71 ± 4 mmHg in control group, $p < 0.05$, Fig. 3B) in the mice, treatment with verteporfin significantly reduced Ang II-induced elevation of SBP (144 ± 3 vs. 165 ± 4 mmHg in Ang II group, $p < 0.05$, Fig. 3A) and DBP (88 ± 4 vs. 102 ± 3 mmHg in Ang II group, $p < 0.05$, Fig. 3B). Ang II also increased the thickness of aortic wall (68.7 ± 1.0 vs. 48.7 ± 2.8 μm in control group, $p < 0.05$), which was significantly reduced in Ang II/ Ve mice (53.9 ± 1.8 vs. 68.7 ± 1.0 μm in control, $p < 0.05$, Fig. 3C&D). As shown in Fig. 3E&F, Masson-trichrome staining showed that the staining area of collagen significantly increased in the aorta of Ang II hypertensive mice, and treatment with verteporfin markedly reduced the positive staining area of collagen in Ang II mice. Consistent with the results of Masson-trichrome staining, immunofluorescence staining showed that Ang II also increased the immunofluorescence intensity of collagen I, which was reduced in Ang II/Ve mice (Fig. 3G&H). We have previously demonstrated that Ang II induces renal fibrosis via the stimulation of TGFβ/Smad2/3 pathway (Zhang et al., 2021). Ang II significantly increased aortic expressions of TGFβ1, collagen I and the ratio of phosphor-Smad3/Smad2/3 but not total Smad2/3, treatment with verteporfin markedly reduced these fibrotic factors (Fig. 4). These results suggest that the activation of YAP/TAZ pathway plays an important role in Ang II-induced cardiac and vascular hypertrophy and fibrosis, thereby contributing to cardiovascular remodeling.

3.3. *Ang II promotes proliferation and fibrotic protein expressions via the activation of YAP/TAZ pathway in VSMCs*

We investigated the effect of Ang II on YAP/TAZ activation in A7R5 VSMCs *in vitro*. We used immunofluorescence staining to determine nuclear fluorescence intensity of YAP and TAZ, as shown in Fig. 5A&B & figures C&D, Ang II significantly increased nuclear fluorescence

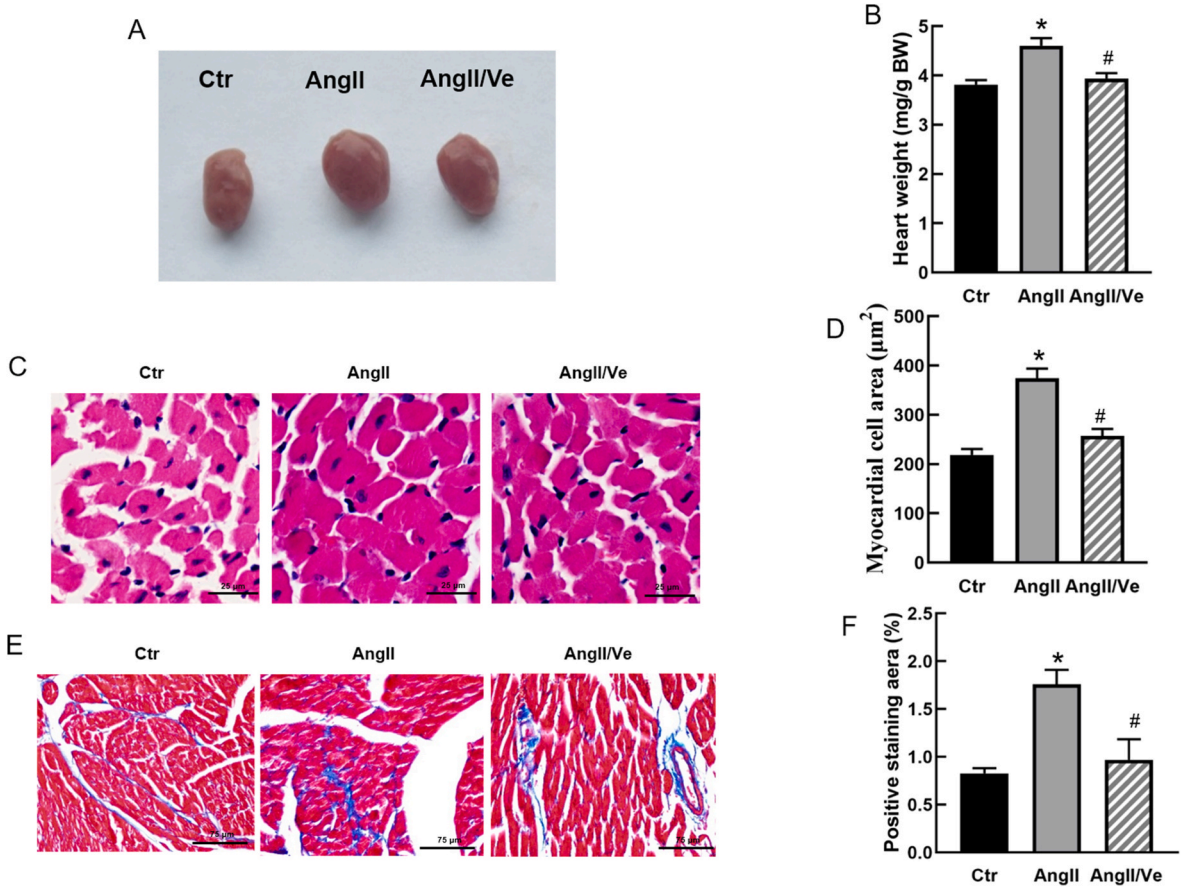


Fig. 2. Treatment with verteporfin reduced Heart weight (A&B), cardiac hypertrophy (C&D), and fibrosis (E&F) in Ang II mice. Representative images of heart section stained with HE (C) or Masson trichrome (E), and the quantitative analysis of cardiomyocyte cross-sectional area (D, bar = 25 μm) and positive collagen stained area (F, bar = 75 μm) in the heart. n = 6 *$p < 0.05$ vs. Ctr group; $^{\#}p < 0.05$ vs. Ang II group.

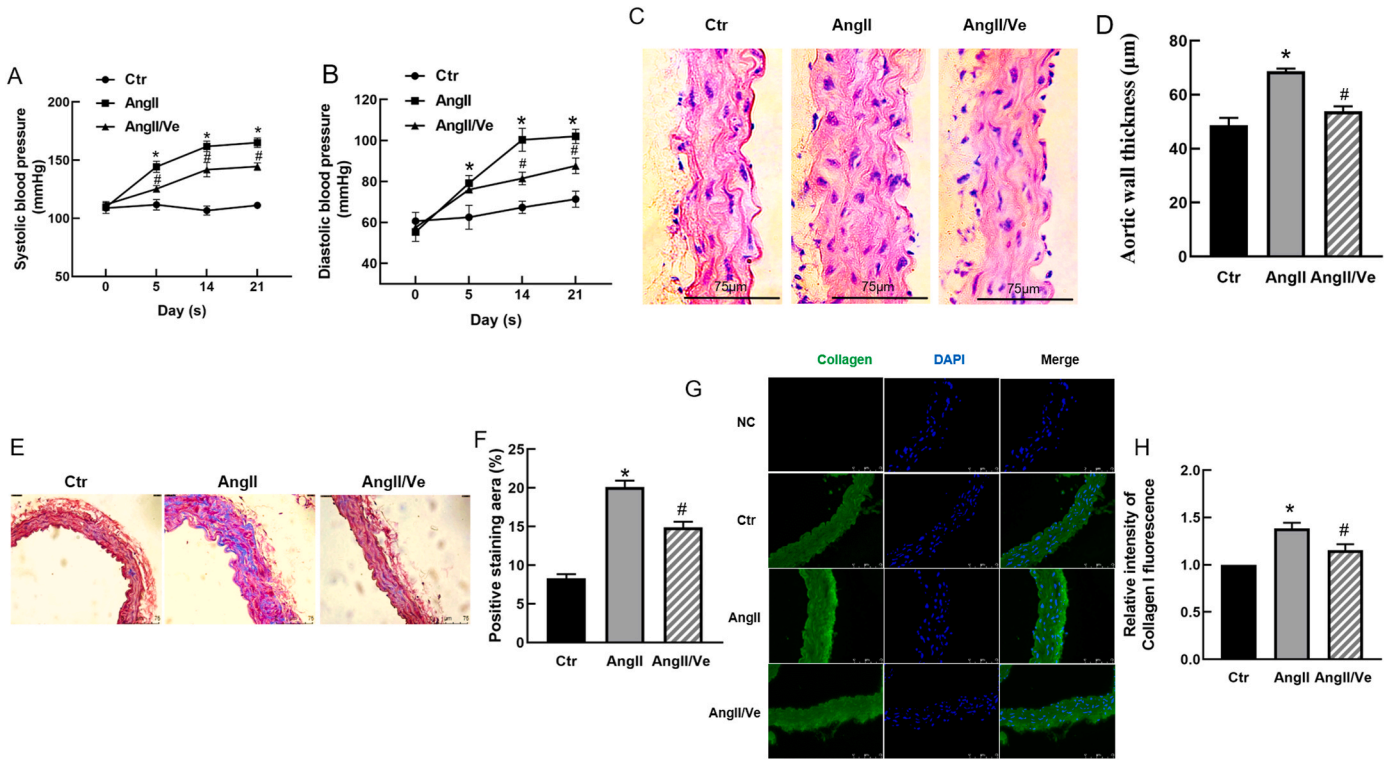


Fig. 3. Verteporfin lowered blood pressure (A&B) and attenuated aortic hypertrophy (C&D) and fibrosis (E&F) in Ang II hypertensive mice. Representative images of the aortic wall stained with HE (C), and quantitative analysis of the aortic wall thickness (D), bar = 75 μm. Representative images of aortic fibrosis determined by Masson trichrome staining (E), and quantitative analysis of positive collage-staining area in aortic section (F). Representative images collagen I in the aorta determined by immunofluorescence (G), bar = 75 μm, quantitative analysis of collagen I fluorescence intensity in the aorta (H). n = 6 *$p < 0.05$ vs. Ctr group; #$p < 0.05$ vs. Ang II group.

intensity of YAP and TAZ, while treatment with verteporfin significantly reduced Ang II-induced increase in the nuclear fluorescence intensity of YAP and TAZ. Ang II time-dependently increased the protein expressions of nuclear YAP and TAZ in VSMCs (Fig. 5E&F), and decreased the expression of cytosolic p-YAP and p-TAZ (Fig. S3). Ang II promoted the proliferation of VSMCs starting from 24 h of treatment (Fig. 5G). Furthermore, Ang II increased the protein expressions of TGFβ1, p-Smad3, fibronectin and collagen I in VSMCs but did not affect the protein expression of total Smad2/3 (Fig. 6). The inhibition of YAP/TAZ activation with verteporfin suppressed Ang II-induced cell proliferation (Fig. 5G) and the expression of these fibrotic proteins (Fig. 6). These results suggest that Ang II activation of the YAP/TAZ pathway help promote VSMC proliferation and fibrosis.

3.4. Ang II activates YAP/TAZ pathway via the stimulation of Rho A/ERK1/2 pathway in VSMCs

We have recently shown that Ang II activates the YAP/TAZ pathway via the activation of Protein Phosphatases 2A subunit C (PP2Ac) dephosphorylation in HUVECs(Xu et al., 2021). In this study, we investigated effect of Ang II on the protein expression of PP2Ac and phosphor-PP2Ac in VSMCs. However, Ang II did not affect the protein expression of PP2Ac and phosphor-PP2Ac (Fig. S4). It has been shown that Ang II activates Rho kinases to induce VSMC proliferation and the expression of fibrotic factors (Du et al., 2015; Wu et al., 2019b). We determined the protein expression of Rho A in Ang II-treated VSMCs. Ang II time-dependently increased Rho A expression (Fig. 7A), and the inhibition of Rho kinases with fasudil prevented Ang II-induced Rho A protein expression (Fig. 7B). To investigate the effect of fasudil on Ang II-induced YAP/TAZ activation, fibrosis and cell proliferation in VSMCs, cells were preincubated with fasudil for 30 min followed by the incubation with Ang II for 24 h. Fasudil prevented Ang II-induced nuclear translocation of YAP and TAZ (Fig. 7C&D), and decreased cytosolic expression of p-YAP and p-TAZ (Fig. S5), fasudil also inhibited Ang II-induced protein expression of TGFβ, p-Smad3, collagen I and cell proliferation (Fig. 7E–I). These results indicate that Ang II-induced YAP/TAZ activation, fibrosis and cell proliferation is mediated by stimulating Rho kinase in VSMCs.

It has been shown that Rho A can activate ERK1/2 and promote the nuclear translocation of p-ERK1/2, which may bind to YAP to stabilize YAP in nucleus (Qin et al., 2019; Zhu et al., 2008). To investigate whether ERK1/2 is a downstream molecule of Rho A to mediate Ang II activation of YAP/TAZ pathway, we investigated the effect of fasudil on Ang II-induced ERK1/2 phosphorylation (the active form of ERK1/2) in A7R5 VSMCs, Ang II significantly increased the expression of p-ERK1/2, which was inhibited by fasudil, suggesting that Rho kinase is upstream molecule of Ang II activation of ERK1/2. Then, we investigated the effect of ERK1/2 inhibitor PD98059 on Ang II-induced YAP/TAZ activation, VSMCs were preincubated with ERK1/2 inhibitor PD98059 for 30 min followed by incubation with Ang II for 24 h, PD98059 effectively inhibited Ang II-induced ERK1/2 phosphorylation (Fig. S6A), the inhibition of ERK1/2 activation with PD98059 prevented an increase in Ang II-induced expression of YAP/TAZ in the nucleus (Fig. 8B&C) and a decrease in Ang II-induced the expression of p-YAP/p-TAZ in the cytoplasm (Fig. S6B&C), while the inhibition of YAP/TAZ with verteporfin did not affect p-ERK1/2 expression (Fig. S7), suggesting that ERK1/2 is upstream molecule of Ang II activation of YAP/TAZ. Finally we investigated the effect of PD98059 on Ang II-induced cell proliferation and fibrotic signaling TGFβ/Smad2/3, PD98059 significantly inhibited Ang II-induced the protein expression of TGFβ (Fig. 8D), p-Smad3 and collagen I (Fig. 8F&G) and cell proliferation (8H), but did not affected the protein expression of total Smad2/3 (Fig. 8E). These results indicate that Ang II activates YAP/TAZ pathway via the stimulation of Rho A/ERK1/2 pathway, the activation of this pathway contributes to Ang

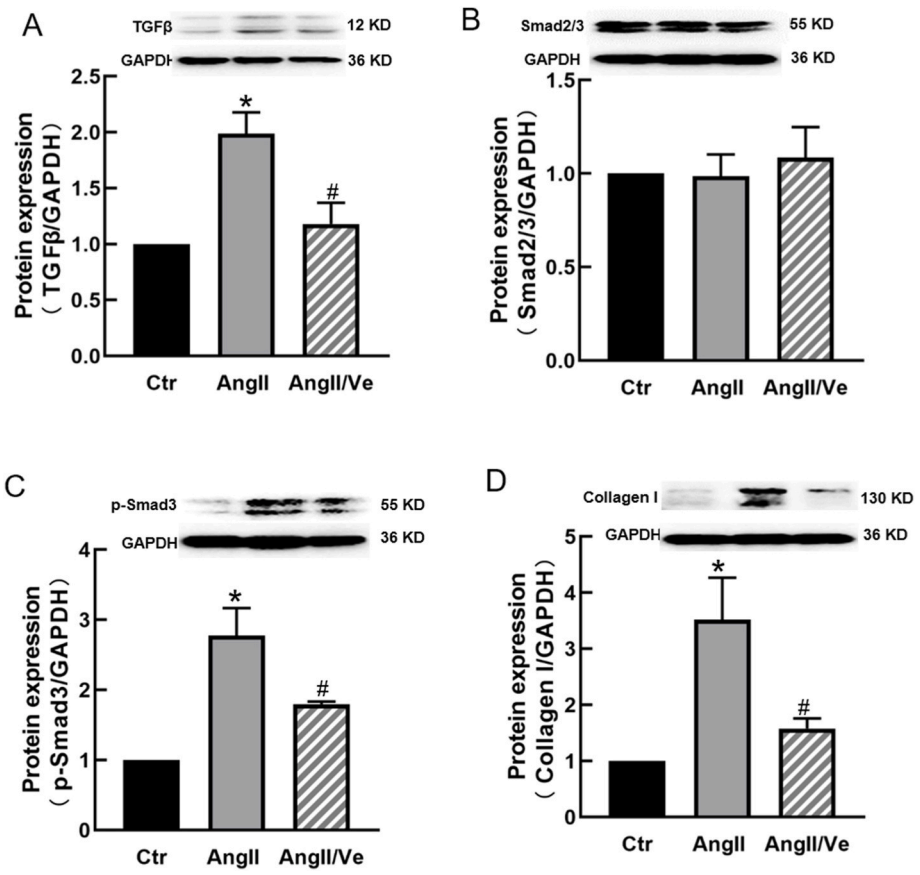


Fig. 4. Treatment with verteporfin prevented Ang II activation of transform growth factor β (TGF-β)/Smad/collage I pathway, the protein expression of TGFβ (A), Smad2/3 (B) and p-Smad3 (C) and collagen I (D). n = 6 *p < 0.05, vs. Ctr group; #p < 0.05 vs. Ang II group.

II-induced VSMC proliferation and fibrosis.

4. Discussion

There is growing evidence showing the importance of the Hippo/YAP pathway in vascular remodeling and related cardiovascular diseases (Mia and Singh, 2022a; Zheng et al., 2022). We have previously shown that Ang II activates the YAP/TAZ pathway in the endothelium, which contributes to endothelial dysfunction and vascular inflammation (Xu et al., 2021). In the present study, we further demonstrate that Ang II activates the YAP/TAZ pathway in the heart and vessels, as an inhibitor of YAP/TAZ activation, verteporfin suppresses Ang II-induced YAP/TAZ activation and prevents Ang II-induced vascular and cardiac hypertrophy and fibrosis. In cultured VSMCs, Ang II activates the Rho A/ERK1/2 pathway to induce YAP/TAZ activation, thereby promoting VSMC proliferation and fibrosis. These results strongly suggest that the YAP/TAZ pathway plays a critical role in Ang II-induced hypertension and vascular remodeling and injury.

YAP/TAZ is the main down effector and transcriptional co-regulator of the Hippo pathway, YAP and TAZ cooperatively regulate the transcription of target genes. The activation of YAP/TAZ is Hippo-dependent and Hippo-independent, and the inactivation of Hippo signaling pathway induces dephosphorylation and activation of YAP/TAZ by initiating cascade dephosphorylations of Hippo downstream molecules, such as LATS1 (large tumor suppressor 1/2) (Zhao et al., 2020). In additional Hippo pathway, other molecules, such as small GTPase proteins, integrins and G protein-coupled receptor mediators have been shown to regulate YAP/TAZ activities through Hippo-independent phosphorylation of YAP/TAZ (Jang et al., 2020; Yamashiro et al., 2020; Zindel et al., 2021).

Recent studies suggest that YAP/TAZ plays an important role for maintenance of vascular homeostasis in health individual and is involved in the regulation of cardiovascular remodeling in cardiovascular diseases (Lv and Ai, 2022; Yamashiro et al., 2020). The Hippo/YAP pathway controls organ size and tissue homeostasis by regulating cell growth, death and differentiation (Xie et al., 2022). Vascular remodeling is an active process that involves the death, proliferation and migration of endothelial cells and VSMCs as well as the production and degradation of ECM, and is the pathological basis of hypertensive vascular complications (Cai et al., 2021). Ang II is an important contributor to hypertensive cardiovascular remodeling (Harrison et al., 2021). Ang II promotes cardiac and vascular inflammation, fibrosis and VSMC proliferation and migration via the facilitation of AT1R-mediated various intracellular signaling pathways (Cantero-Navarro et al., 2021; Huynh et al., 2022). We found that Ang II induced cardiovascular remodeling via the activation of YAP/TAZ pathway, because the inhibition of YAP/TAZ by verteporfin attenuated Ang II-induced cardiac and vascular hypertrophy and fibrosis.

Ang II can activate multiple signaling pathways, including YAP/TAZ, ERK1/2, Rho A/ROCK kinase (Ohtsu et al., 2005). RhoA/ROCK kinase is the final pathway of a wide spectrum of molecular effectors such as Angiotensin II, and participates in regulating cell division, the actin-based cytoskeleton, reactive oxygen species genesis and VSMC constriction (Li et al., 2019; Nour-Eldine et al., 2016). It has been shown that RhoA/ROCK kinase is essential for Ang II-induced cardiovascular remodeling, and the blockade of Rho A by lovastatin inhibits Ang II-induced YAP/TAZ activation and vascular fibrosis in mice (Wu et al., 2019a). ERK1/2 is an important signaling molecule to regulate cell proliferation and cardiac and vascular hypertrophy, it has been shown that Rho A can stimulate ERK1/2 phosphorylation and the nuclear translocation of p-ERK1/2 to bind to and stabilize YAP in the nucleus (Hao et al., 2022). In the present study, we found that Ang II promoted

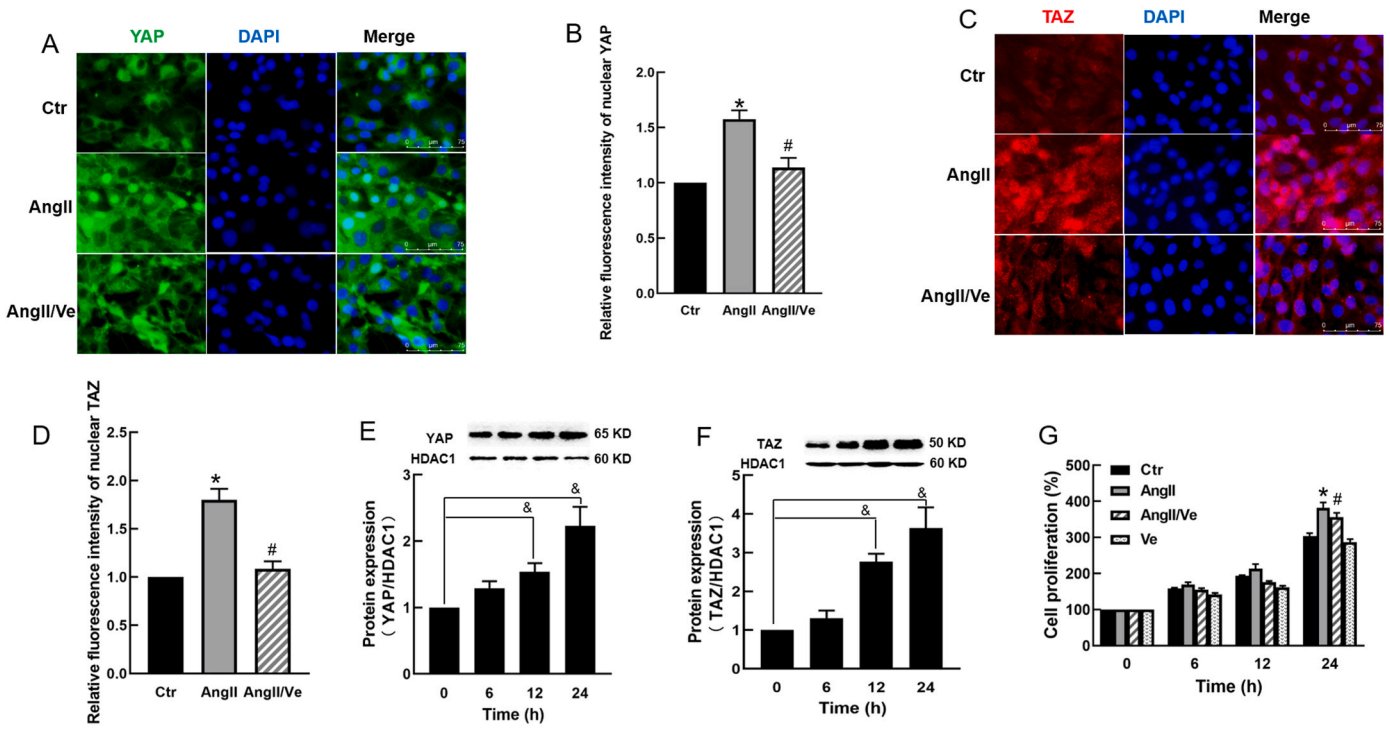


Fig. 5. Ang II induced YAP/TAZ activation in A7R5 vascular smooth muscle cells (VSMCs). Representative images of YAP (A) and TAZ (C) expressions in the nucleus of VSMCs, determined by immunofluorescence staining, YAP was immunolabeled with FITC (Green) , cell nucleus stained with DAPI (blue), bar = 75 μm. Quantitative analysis of YAP (B) and TAZ (D) fluorescence intensity in the nucleus of A7R5 VSMCs. Ang II time-dependently increased the protein expression of YAP (E) and TAZ (F) in the nuclear fraction of VSMCs; verteporfin prevented Ang II-induced VSMC proliferation (G). n = 6 $^{\&}$ $p < 0.05$; *$p < 0.05$ vs. Ctr group; $^{\#}p < 0.05$ vs. Ang II group.

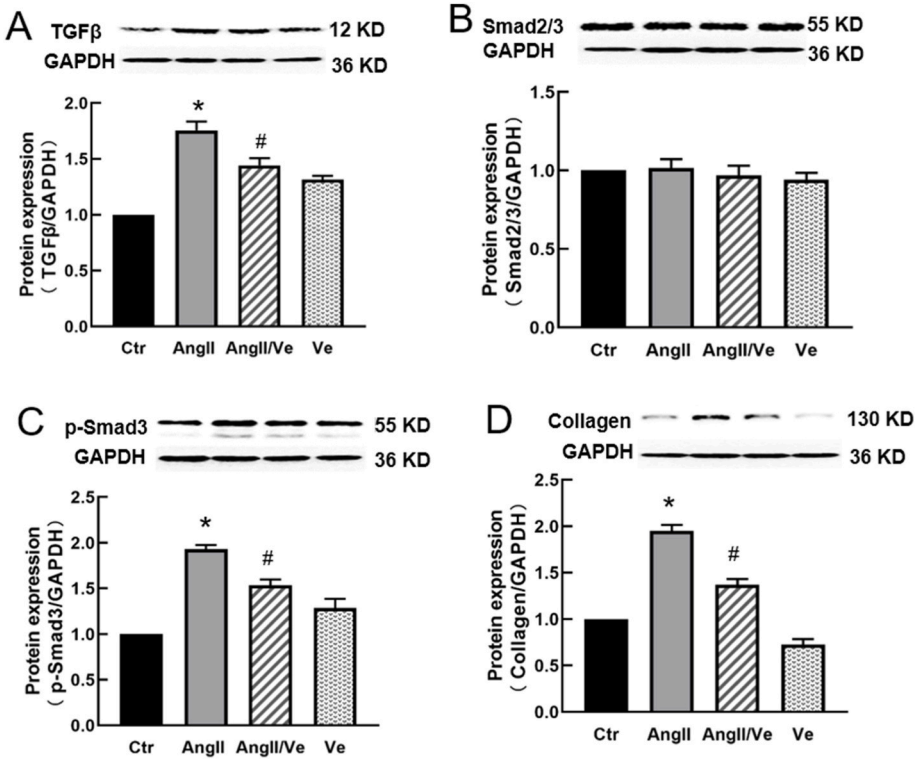


Fig. 6. Verteporfin prevented Ang II activation of TGFβ/Smad2/3 signaling in VSMCs. The protein expression of transform growth factor β (TGF-β, A), phosphor-Smad3 (B), total Smad2/3 (C) and collagen I (D) . n = 3 *$p < 0.05$ vs. Ctr group; $^{\#}p < 0.05$ vs. Ang II group.

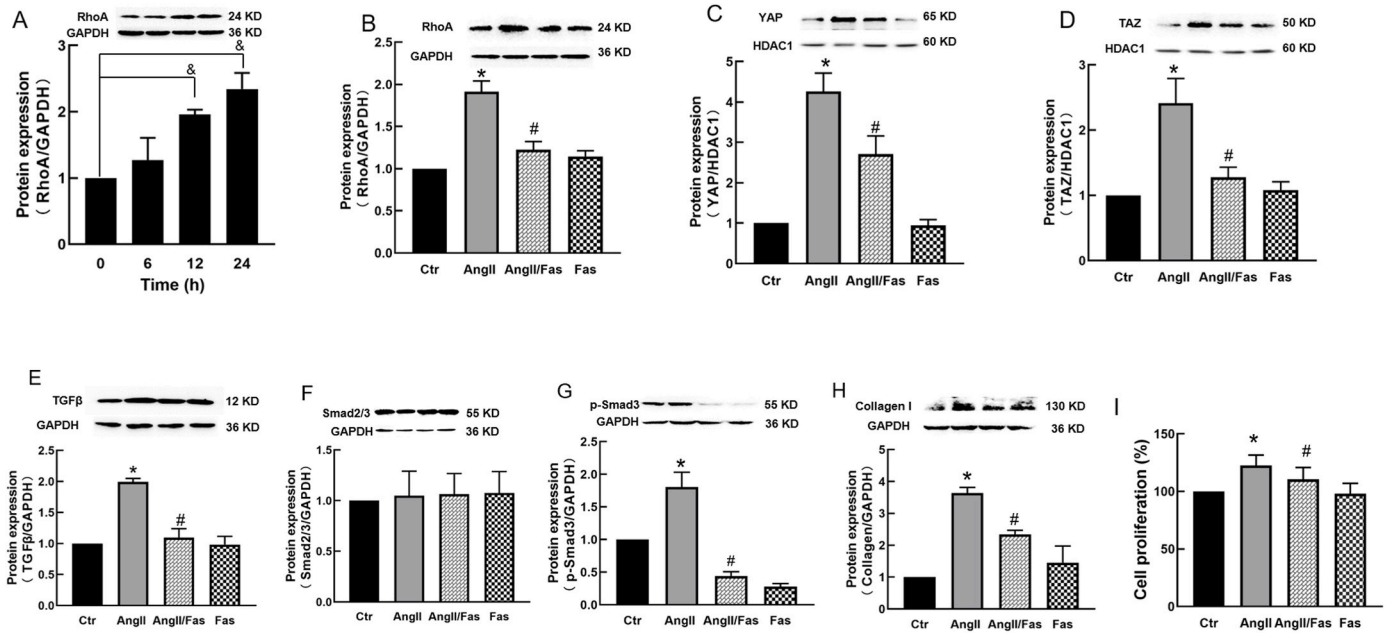


Fig. 7. Ang II stimulated Rho A to promote YAP/TAZ activation in A7R5 VSMCs. Ang II increased the protein expression of Rho A (A) in a time-dependent way; Fasudil (Fas, a Rho-associated protein kinase inhibitor, 10 μM) inhibited Ang II-induced expression of RhoA (B). Fasudil inhibited Ang II-induced YAP/TAZ nuclear translocation (C&D), the protein expressions of YAP (C) and TAZ (D) in the nuclear fraction. Fasudil prevented Ang II activation of TGFβ/Smad2/3 signaling, the protein expression of TGFβ (E), total Smad2/3 (F), p-Smad3 (G) and collagen I (H). Fasudil prevented Ang II-induced VSMC proliferation (I). n = 3 $^{\&}p < 0.05$; $^{\&}p < 0.05$, $^{*}p < 0.05$ vs. Ctr group, $^{\#}p < 0.05$ vs. Ang II group.

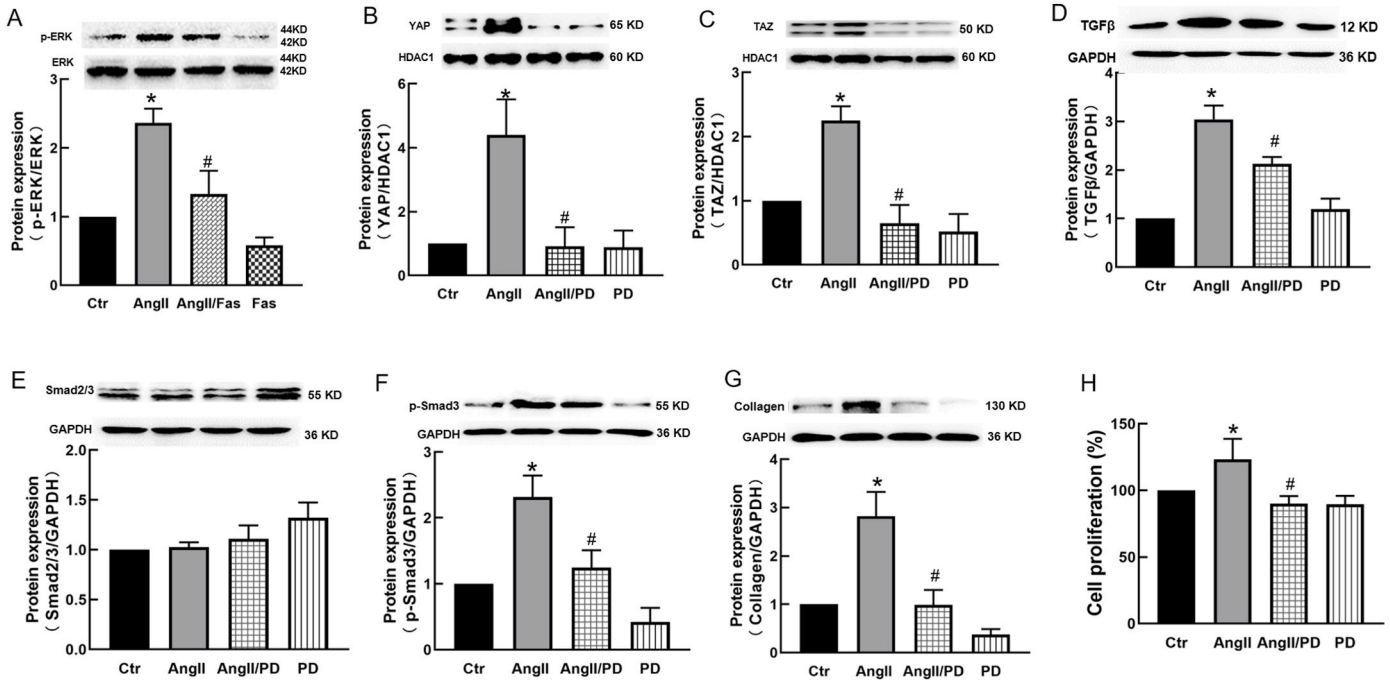


Fig. 8. Ang II activated the YAP/TAZ pathway via stimulating Rho A/ERK1/2 signaling in A7R5 VSMCs. Inhibition of Rho kinase with fasudil suppressed Ang II-induced p-ERK expression (A), ERK inhibitor PD98059 decreased the nuclear expressions of YAP (B) and TAZ (C); PD98059 prevented Ang II activation of TGFβ/Smad2/3 signaling, the protein expression of TGFβ (D), total Smad2/3 (E), p-Smad3 (F) and collagen I (G). PD98059 prevented Ang II-induced VSMC proliferation (H). n = 3 $^{*}p < 0.05$ vs. Ctr group; $^{\#}p < 0.05$ vs. Ang II group.

YAP/TAZ activation in the aorta and heart of mice and in cultured VSMC, Ang II also increased the expression of Rho A and p-ERK1/2, and either Rho kinase inhibitor fasudil or ERK1/2 inhibitor PD98059 inactivated YAP/TAZ in VSMCs and inhibited Ang II-induced cell proliferation and fibrotic protein expressions. These results suggest that Ang II activates YAP/TAZ pathway through the activating the Rho A/ERK1/2 pathway. Because fasudil inhibited ERK1/2 activation, and verteporfin did not affect p-ERK1/2 expression, thereby we surmise that Rho kinase is upstream molecule of Ang II activation of ERK1/2, which subsequently activates the YAP/TAZ pathway to promote VSMC proliferation and expression of fibrotic proteins.

Recently, we have shown that Ang II activates YAP/TAZ through

PP2Ac-dependent dephosphorylation of YAP/TAZ in the endothelium, which contributes to endothelial activation and vascular inflammation (Xu et al., 2021). However, PP2Ac inhibitor LB-100 did not inhibit Ang II-induced YAP/TAZ activation in VSMCs. Thus, it seems that Ang II activates YAP/TAZ through different signaling pathways in the endothelium and VSMCs. Ang II activates YAP/TAZ in the endothelium through YAP/TAZ and in VSMCs through Rho A/ERK1/2, which may regulate different cell fate in the endothelium and VSMCs.

Fibrotic response is a critical contributor to hypertensive end organ damage and cardiovascular remodeling (Intengan and Schiffrin, 2001). It has been shown that YAP/TAZ plays a critical role in regulating fibrotic responses in various type of cells, and YAP/TAZ promotes tissue and organ fibrogenesis through the activation of the TGFβ/Smad2/3 pathway (Mia and Singh, 2022b; Varelas et al., 2010). The inhibition of YAP/TAZ attenuates unilateral ureteral obstruction-induced renal fibrosis associated suppressing TGFβ/Smad2/3 pathway (Szeto et al., 2016). In this study, we found that verteporfin inhibited Ang II-induced vascular fibrosis and the expression of profibrotic factors TGFβ and collagen I in VSMCs, suggesting that Ang II induces vascular fibrosis through stimulating YAP/TAZ-mediated TGFβ/Smad2/3 pathway.

Limitations: There are several limitations in our study. First, it is well known that high blood pressure is an important factor to induce cardiac and vascular hypertrophy and remodeling, and Ang II-induced cardiac and vascular hypertrophy and remodeling in hypertensive mice are blood pressure-dependent and blood pressure-independent (Salah et al., 2019). In this study, verteporfin mildly lowered blood pressure in Ang II hypertensive mice, so it can't be ruled out that decrease in SBP itself may help reduce cardiac and vascular hypertrophy and remodeling in Ang II/Ve mice. However, blood pressure in Ang II/Ve mice remained hypertension, while verteporfin significantly lowered aortic and heart weight as well as fibrosis. Furthermore, our data in *in vitro* VSMCs showed that verteporfin can directly inhibit Ang II-induced VSMC proliferation and fibrotic TGFβ1/Smad2/3 signaling. Therefore, we believe that the activation of YAP/TAZ is the main factor leading to cardiovascular remodeling and hypertrophy in Ang II hypertension. Next, although we showed that Ang II induced the activation of the YAP/TAZ pathway, cell proliferation and fibrosis in VSMCs through stimulating the Rho A/ERK1/2 pathway, it is still unclear whether Ang II induces YAP/TAZ activation and proliferation and fibrosis in myocardial cells through the similar signaling mechanisms. Clarifying these mechanisms in myocardial cells and heart tissue will be our future study. Finally, we did not have verteporfin alone in normal mice, which may help us understand verteporfin's effects in normal mice. However, verteporfin did not significantly affect the parameters in VSMCs, thus we believe that the results in absence of this group should not affect our conclusion.

In conclusion, in the present study, we provide solid evidence to demonstrate that Ang II activates YAP/TAZ to induce hypertensive cardiac and vascular remodeling and VSMC proliferation and fibrosis. Ang II activation of YAP/TAZ is mediated by Rho A/ERK1/2 pathway in VSMCs. YAP/TAZ may be a new target for hypertensive and cardiovascular diseases.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 81970357, 82270434) to MSZ; Basic Scientific Research Project of Education Department of Liaoning Province (LJKMZ20221800) to QX.

CRediT authorship contribution statement

Qian Xu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision. **Kunping Zhuo:** Data curation, Formal analysis, Investigation, Methodology, Validation. **Xiaotian Zhang:** Data curation, Investigation, Methodology. **Yanru Zhen:** Data curation, Investigation, Methodology. **Limin Liu:** Data curation, Investigation, Methodology. **Lu Zhang:** Data curation, Investigation, Methodology. **Yufan Gu:** Data curation, Investigation, Methodology. **Hui Jia:** Data curation, Investigation, Methodology. **Qing Chen:** Investigation, Methodology. **Meixi Liu:** Investigation, Methodology. **Jiawei Dong:** Investigation, Methodology. **Ming-Sheng Zhou:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.
original data and Supplementary data (Original data) (Figshare)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2023.176252.

References

Ashour, L., 2022. Roles of the ACE/Ang II/AT1R pathway, cytokine release, and alteration of tight junctions in COVID-19 pathogenesis. Tissue Barriers 11, 2090792.
Bregeon, J., Loirand, G., Pacaud, P., Rolli-Derkinderen, M., 2009. Angiotensin II induces RhoA activation through SHP2-dependent dephosphorylation of the RhoGAP p190A in vascular smooth muscle cells. Am. J. Physiol.: Cell Physiol. 297, C1062–C1070.
Cai, Z., Gong, Z., Li, Z., Li, L., Kong, W., 2021. Vascular extracellular matrix remodeling and hypertension. Antioxidants Redox Signal. 34, 765–783.
Cantero-Navarro, E., Fernandez-Fernandez, B., Ramos, A.M., Rayego-Mateos, S., Rodrigues-Diez, R.R., Sanchez-Nino, M.D., Sanz, A.B., Ruiz-Ortega, M., Ortiz, A., 2021. Renin-angiotensin system and inflammation update. Mol. Cell. Endocrinol. 529, 111254.
Du, C.Q., Liu, X.W., Zeng, G.Z., Jin, H.F., Tang, L.J., 2015. Inhibition of farnesyl pyrophosphate synthase attenuates angiotensin II-induced fibrotic responses in vascular smooth muscle cells. Int. J. Mol. Med. 35, 1767–1772.
Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S., Elvassore, N., Piccolo, S., 2011. Role of YAP/TAZ in mechanotransduction. Nature 474, 179–183.
Gallo, G., Volpe, M., Savoia, C., 2021. Endothelial dysfunction in hypertension: current concepts and clinical implications. Front. Med. 8, 798958.
Hao, X., Shi, B., Li, W., Wu, Y., Ai, N., Zhu, L., Wu, Z., Li, Z., 2022. Fasudil inhibits hepatic artery spasm by repressing the YAP/ERK/ ET(A)/ET(B) signaling pathway via inhibiting ROCK activation. Aging 14, 7378–7389.
Harrison, C.B., Trevelin, S.C., Richards, D.A., Santos, C.X.C., Sawyer, G., Markovinovic, A., Zhang, X., Zhang, M., Brewer, A.C., Yin, X., Mayr, M., Shah, A.M., 2021. Fibroblast Nox2 (NADPH oxidase-2) regulates ANG II (angiotensin II)-Induced vascular remodeling and hypertension via paracrine signaling to vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 41, 698–710.
Huang, L., Wang, A., Hao, Y., Li, W., Liu, C., Yang, Z., Zheng, F., Zhou, M.S., 2018. Macrophage depletion lowered blood pressure and attenuated hypertensive renal injury and fibrosis. Front. Physiol. 9, 473.
Humphrey, J.D., 2021. Mechanisms of vascular remodeling in hypertension. Am. J. Hypertens. 34, 432–441.
Huynh, D.T.N., Jin, Y., Van Nguyen, D., Myung, C.S., Heo, K.S., 2022. Ginsenoside Rh1 inhibits angiotensin II-induced vascular smooth muscle cell migration and proliferation through suppression of the ROS-mediated ERK1/2/p90RSK/KLF4 signaling pathway. Antioxidants 11, 643.
Intengan, H.D., Schiffrin, E.L., 2001. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. Hypertension 38, 581–587.
Jang, J.W., Kim, M.K., Bae, S.C., 2020. Reciprocal regulation of YAP/TAZ by the Hippo pathway and the Small GTPase pathway. Small GTPases 11, 280–288.
Kawai, T., Forrester, S.J., O'Brien, S., Baggett, A., Rizzo, V., Eguchi, S., 2017. AT1 receptor signaling pathways in the cardiovascular system. Pharmacol. Res. 125, 4–13.
Kwon, H., Kim, J., Jho, E.H., 2022. Role of the Hippo pathway and mechanisms for controlling cellular localization of YAP/TAZ. FEBS J. 289, 5798–5818.
Li, Q., Wo, D., Huang, Y., Yu, N., Zeng, J., Chen, H., Wang, H., Bao, L., Lin, S., Chu, J., Peng, J., 2019. Alkaloids from Nelumbinis Plumula (AFNP) ameliorate aortic remodeling via RhoA/ROCK pathway. Biomed. Pharmacother. 112, 108651.
Li, R., Huang, W., 2023. Yes-associated protein and transcriptional coactivator with PDZ-binding motif in cardiovascular diseases. Int. J. Mol. Sci. 24, 1666.

Lin, M., Yuan, W., Su, Z., Lin, C., Huang, T., Chen, Y., Wang, J., 2018. Yes-associated protein mediates angiotensin II-induced vascular smooth muscle cell phenotypic modulation and hypertensive vascular remodelling. Cell Prolif. 51, e12517.
Liu, D., Lv, H., Liu, Q., Sun, Y., Yu, B., 2019. Atheroprotective effects of methotrexate via the inhibition of YAP/TAZ under disturbed flow. J. Transl. Med. 17, 378.
Liu, H., Ning, F., Lash, G.E., 2022. Contribution of vascular smooth muscle cell apoptosis to spiral artery remodeling in early human pregnancy. Placenta 120, 10–17.
Lv, H., Ai, D., 2022. Hippo/yes-associated protein signaling functions as a mechanotransducer in regulating vascular homeostasis. J. Mol. Cell. Cardiol. 162, 158–165.
Martyniak, A., Tomasik, P.J., 2022. A new perspective on the renin-angiotensin system. Diagnostics 13, 16.
Mia, M.M., Singh, M.K., 2022a. Emerging roles of the Hippo signaling pathway in modulating immune response and inflammation-driven tissue repair and remodeling. FEBS J. 289, 4061–4081.
Mia, M.M., Singh, M.K., 2022b. New insights into hippo/YAP signaling in fibrotic diseases. Cells 2065, 11.
Moya, I.M., Halder, G., 2019. Hippo-YAP/TAZ signalling in organ regeneration and regenerative medicine. Nat. Rev. Mol. Cell Biol. 20, 211–226.
Nour-Eldine, W., Ghantous, C.M., Zibara, K., Dib, L., Issaa, H., Itani, H.A., El-Zein, N., Zeidan, A., 2016. Adiponectin attenuates angiotensin II-induced vascular smooth muscle cell remodeling through nitric oxide and the RhoA/ROCK pathway. Front. Pharmacol. 7, 86.
Ohtsu, H., Mifune, M., Frank, G.D., Saito, S., Inagami, T., Kim-Mitsuyama, S., Takuwa, Y., Sasaki, T., Rothstein, J.D., Suzuki, H., Nakashima, H., Woolfolk, E.A., Motley, E.D., Eguchi, S., 2005. Signal-crosstalk between Rho/ROCK and c-Jun NH2-terminal kinase mediates migration of vascular smooth muscle cells stimulated by angiotensin II. Arterioscler. Thromb. Vasc. Biol. 25, 1831–1836.
Pocaterra, A., Romani, P., Dupont, S., 2020. YAP/TAZ functions and their regulation at a glance. J. Cell Sci. 133, jcs230425.
Qin, X., Li, J., Sun, J., Liu, L., Chen, D., Liu, Y., 2019. Low shear stress induces ERK nuclear localization and YAP activation to control the proliferation of breast cancer cells. Biochem. Biophys. Res. Commun. 510, 219–223.
Salah, E., Bastacky, S.I., Jackson, E.K., Tofovic, S.P., 2019. 2-Methoxyestradiol attenuates angiotensin II-induced hypertension, cardiovascular remodeling, and renal injury. J. Cardiovasc. Pharmacol. 73, 165–177.
Slivnick, J., Lampert, B.C., 2019. Hypertension and heart failure. Heart Fail. Clin. 15, 531–541.
Szeto, S.G., Narimatsu, M., Lu, M., He, X., Sidiqi, A.M., Tolosa, M.F., Chan, L., De Freitas, K., Bialik, J.F., Majumder, S., Boo, S., Hinz, B., Dan, Q., Advani, A., John, R., Wrana, J.L., Kapus, A., Yuen, D.A., 2016. YAP/TAZ are mechanoregulators of TGF-beta-smad signaling and renal fibrogenesis. J. Am. Soc. Nephrol. : JASN (J. Am. Soc. Nephrol.) 27, 3117–3128.
Varelas, X., Samavarchi-Tehrani, P., Narimatsu, M., Weiss, A., Cockburn, K., Larsen, B.G., Rossant, J., Wrana, J.L., 2010. The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway. Dev. Cell 19, 831–844.
Wang, L., Luo, J.Y., Li, B., Tian, X.Y., Chen, L.J., Huang, Y., Liu, J., Deng, D., Lau, C.W., Wan, S., Ai, D., Mak, K.K., Tong, K.K., Kwan, K.M., Wang, N., Chiu, J.J., Zhu, Y., Huang, Y., 2016. Integrin-YAP/TAZ-JNK cascade mediates atheroprotective effect of unidirectional shear flow. Nature 540, 579–582.
Wei, H., Wang, F., Wang, Y., Li, T., Xiu, P., Zhong, J., Sun, X., Li, J., 2017. Verteporfin suppresses cell survival, angiogenesis and vasculogenic mimicry of pancreatic ductal adenocarcinoma via disrupting the YAP-TEAD complex. Cancer Sci. 108, 478–487.
Wu, P., Liu, Z., Zhao, T., Xia, F., Gong, L., Zheng, Z., Chen, Z., Yang, T., Duan, Q., 2019a. Lovastatin attenuates angiotensin II induced cardiovascular fibrosis through the suppression of YAP/TAZ signaling. Biochem. Biophys. Res. Commun. 512, 736–741.
Wu, Y., Li, Z., Wang, S., Xiu, A., Zhang, C., 2019b. Carvedilol inhibits angiotensin II-induced proliferation and contraction in hepatic stellate cells through the RhoA/rho-kinase pathway. Biomed Res. Int. 2019, 7932046.
Xie, J., Wang, Y., Ai, D., Yao, L., Jiang, H., 2022. The role of the Hippo pathway in heart disease. FEBS J. 289, 5819–5833.
Xu, Q., Zhuo, K., Cai, R., Su, X., Zhang, L., Liu, Y., Zhu, L., Ren, F., Zhou, M.S., 2021. Activation of yes-associated protein/PDZ-binding motif pathway contributes to endothelial dysfunction and vascular inflammation in AngiotensinII hypertension. Front. Physiol. 12, 732084.
Yamashiro, Y., Thang, B.Q., Ramirez, K., Shin, S.J., Kohata, T., Ohata, S., Nguyen, T.A.V., Ohtsuki, S., Nagayama, K., Yanagisawa, H., 2020. Matrix mechanotransduction mediated by thrombospondin-1/integrin/YAP in the vascular remodeling. Proc. Natl. Acad. Sci. U.S.A. 117, 9896–9905.
Yildiz, M., Oktay, A.A., Stewart, M.H., Milani, R.V., Ventura, H.O., Lavie, C.J., 2020. Left ventricular hypertrophy and hypertension. Prog. Cardiovasc. Dis. 63, 10–21.
Zhang, J., Xu, Q., Ren, F., Liu, Y., Cai, R., Yao, Y., Zhou, M.S., 2021. Inhibition of YAP activation attenuates renal injury and fibrosis in angiotensin II hypertensive mice. Can. J. Physiol. Pharmacol. 99, 1000–1006.
Zhao, C., Zeng, C., Ye, S., Dai, X., He, Q., Yang, B., Zhu, H., 2020. Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding motif (TAZ): a nexus between hypoxia and cancer. Acta Pharm. Sin. B 10, 947–960.
Zhao, Y., Shang, F., Shi, W., Zhang, J., Zhang, J., Liu, X., Li, B., Hu, X., Wang, L., 2019. Angiotensin II receptor type 1 antagonists modulate vascular smooth muscle cell proliferation and migration via AMPK/mTOR. Cardiology 143, 1–10.
Zheng, A., Chen, Q., Zhang, L., 2022. The Hippo-YAP pathway in various cardiovascular diseases: focusing on the inflammatory response. Front. Immunol. 13, 971416.
Zhu, S., Korzh, V., Gong, Z., Low, B.C., 2008. RhoA prevents apoptosis during zebrafish embryogenesis through activation of Mek/Erk pathway. Oncogene 27, 1580–1589.
Zindel, D., Mensat, P., Vol, C., Homayed, Z., Charrier-Savournin, F., Trinquet, E., Baneres, J.L., Pin, J.P., Pannequin, J., Roux, T., Dupuis, E., Prezeau, L., 2021. G protein-coupled receptors can control the Hippo/YAP pathway through Gq signaling. FASEB J. 35, e21668.