Life Sciences 233 (2019) 116748

Contents lists available at ScienceDirect

Life Sciences

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

Resveratrol prevents chronic intermittent hypoxia-induced cardiac hypertrophy by targeting the PI3K/AKT/mTOR pathway

Peng Guan[1], Zhi-Min Sun[1], Na Wang, Jian Zhou, Li-Fei Luo, Ya-Shuo Zhao, En-Sheng Ji*

Department of Physiology, Hebei University of Chinese Medicine, Shijiazhuang, Hebei, China

ARTICLE INFO

Keywords:
Resveratrol
Chronic intermittent hypoxia
Cardiac hypertrophy
mTOR

ABSTRACT

Aims: Resveratrol is a polyphenolic compound that has received much attention for its use in ameliorating various systemic pathological conditions. The present study was performed to investigate whether the resveratrol alleviated cardiac hypertrophy and functional remodelling by regulating autophagy.

Materials and methods: Male rats were exposed to CIH 8 h/day for five weeks and/or intragastric administration of resveratrol daily. The morphological and echocardiography were used to evaluate the cardiac protective effects. The apoptosis was detected by TUNEL staining. The biochemical assessments were used to evaluate oxidative stress. Further, the effect of resveratrol on autophagy and PI3K/AKT/mTOR pathway was investigated.

Key findings: The CIH group exhibited increased heart weight/body weight and left ventricle weight/body weight ratios, which was accompanied by left ventricular remodelling. Echocardiography analysis showed that CIH-treated rats had significantly higher left ventricular posterior wall thickness, ejection fraction and fractional shortening than those of controls. In addition, the apoptosis index and oxidative markers were significantly elevated in the CIH group versus the control. The autophagy marker Beclin-1 was elevated, while p62 was decreased by CIH treatment. Resveratrol treatment significantly improved cardiac function and alleviated cardiac hypertrophy, oxidative stress, and apoptosis in CIH rats. Further results indicated that PI3K/AKT pathway-mediated inhibition of the mammalian target of rapamycin (mTOR) pathway played a role in the activation of autophagy by resveratrol after CIH stimulation.

Significance: In conclusion, resveratrol supplementation during CIH upregulates autophagy by targeting the PI3K/AKT/mTOR pathway, which appears to be beneficial for resisting cardiac hypertrophy.

1. Introduction

Obstructive sleep apnoea (OSA), with the associated chronic intermittent hypoxia (CIH), is a disorder in which the upper airways relax and collapse during sleep to interrupt normal breathing. Untreated sleep apnoea can lead to increased cardiovascular abnormalities, such as hypertension [20], heart failure [1], atrial fibrillation [10], arrhythmia [34] and coronary artery disease [7]. Additionally, recent studies have shown that OSA is an independent risk factor for severe cardiovascular disease [40]. The pathomechanism of OSA-related cardiovascular injury includes sustained sympathetic activation, vascular endothelial dysfunction, hypercapnia, cytokine dysregulation, systemic inflammation, oxidative stress and metabolic dysregulation [8]. Continuous positive airway pressure (CPAP) treatment is reported to improve left ventricular ejection fraction and reduce systolic blood pressure [26]. However, previous studies have also reported that CPAP may improve sleep metrics and the mental component quality of life but could not reduce cardiovascular disease in patients with OSA [4]. Thus, more effective therapies are needed to improve survival and prevent cardiovascular disease in adults with OSA.

Cardiac hypertrophy has been considered to be independently associated with OSA [29]. Pathological cardiac hypertrophy characterized by increased cardiomyocyte size and accelerated rate of global protein synthesis continues to be a global public health problem leading to contractile dysfunction, arrhythmia, and sudden death. The activation of mammalian target of rapamycin (mTOR) signalling is both indispensable and sufficient for an increase in protein synthesis and the hypertrophy that occurs in response to external stimuli [39]. mTOR is a short-term energy sensor of both amino acids and glucose for the rapid regulation of autophagy [23]. Blocking mTOR signalling can induce autophagy and is beneficial for the heart in order to reduce energy expenditure and misfolded protein accumulation [28]. mTOR

* Corresponding author at: Department of Physiology, Hebei University of Chinese Medicine, Luqian Xingyuan Road 3, Shijiazhuang, Hebei 050200, China.
E-mail address: jesphy@126.com (E.-S. Ji).
[1] Authors contributed equally to this work.

https://doi.org/10.1016/j.lfs.2019.116748
Received 2 May 2019; Received in revised form 2 August 2019; Accepted 10 August 2019
Available online 11 August 2019
0024-3205/

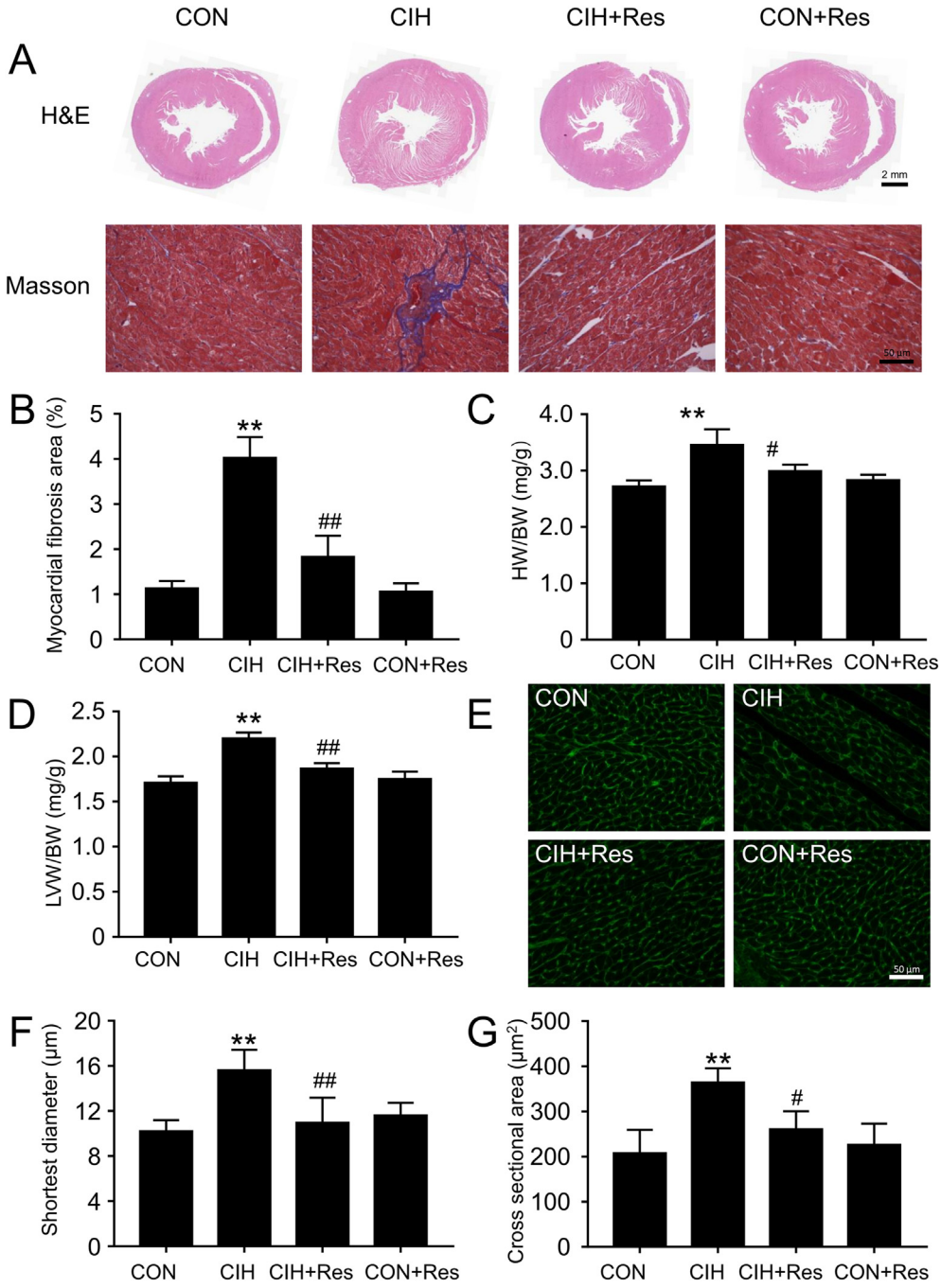

Fig. 1. Histomorphometric data. A. Representative images of H&E and Masson's trichrome stained sections. B. Myocardial fibrosis area. C. HW/BW ratios. D. LVW/BW ratios. E. Representative images of WGA-stained sections. F. The shortest diameters of myocardiocytes. G. Cross-sectional areas of myocardiocytes. $n = 6$. $^{**}p < 0.01$ vs. control group; $^{\#}p < 0.05$ vs. CIH group; $^{\#\#}p < 0.01$ vs. CIH group.

inhibition was also shown to reduce age-related heart inflammation and fibrosis [30]. These results indicate that pharmacological inhibition of mTOR signalling can reactivate cardiac autophagy and may be a viable target for therapy of CIH-induced cardiac injury.

Resveratrol is a polyphenolic compound with excellent antioxidant, anti-inflammatory, insulin-sensitizing, and anti-senescence properties [9]. Significant concentrations of resveratrol have been detected in grapes, blueberries, bilberries, peanuts and in the fruits of other plant species [15]. Several studies have indicated that resveratrol plays an antioxidant role by activating autophagy. Sabe et al. [27] reported that resveratrol prevented harmful cardiac remodelling in chronically ischaemic myocardium through the induction of autophagy. Gu et al. [12] showed that resveratrol-induced autophagy attenuated doxorubicin-induced cardiotoxicity. Resveratrol has also been suggested to improve cardiomyopathy in Duchenne muscular dystrophy mice by ameliorating autophagic flux [18]. Based on these findings, our aim is to investigate whether the resveratrol alleviated cardiac hypertrophy and functional remodelling by regulating autophagy.

2. Materials and methods

2.1. Animals

All animal procedures and experimental protocols were approved by the Hebei University of Chinese Medicine Animal Care and Use Committee. Male SD rats (Beijing Vital River Company, Beijing, China)

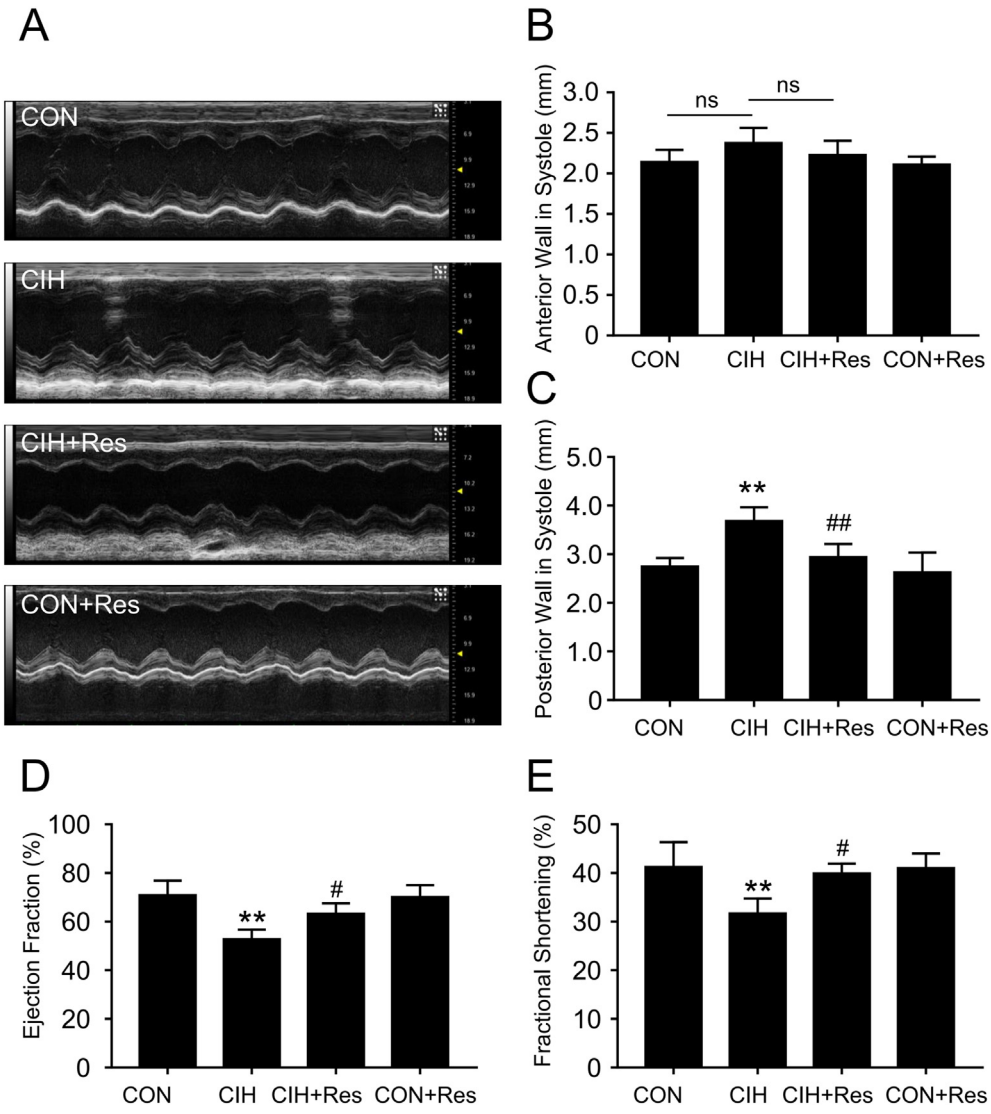

Fig. 2. Effects of resveratrol on LV function assessed by echocardiography in CIH rats. A. Representative recordings obtained from rats following exposure to CIH and/or resveratrol. B. There was no significant change in LV anterior wall thickness in systole among the groups. C. Treatment with resveratrol _ modified the CIH-induced LV posterior wall thickness increase. D. Resveratrol administration preserved contractile function as evidenced by improved ejection fraction. E. Similarly, resveratrol improved fractional shortening. n = 6. $^{**}p < 0.01$ vs. control group; $^{\#}p < 0.05$ vs. CIH group; $^{\#\#}p < 0.01$ vs. CIH group; ns, not significant.

weighing 200–210 g were used to perform all experiments. Rats were given food and water ad libitum.

Rats were randomly assigned to four groups: the control (CON) group, CIH group, CIH + resveratrol (CIH + Res) group, or CON + Res group. CIH rats were placed daily in hypoxia chambers as described previously [13]. Hypoxic chambers (OxyCycler, BioSpherix Ltd., USA) were used to establish OSA rat models. In the chambers, the O_2 concentration was reduced from 21% to 9% by pure N_2 injection, followed by 15 s at 9% O_2, and then O_2 was increased to 21% for 90 s. The cycle was repeated for 8 h daily for five weeks. A similar sham exposure was performed for rats in the CON and Res groups. Both the CIH + Res and CON + Res groups were treated by gavage with Res at a dose of 30 mg/kg body weight daily for five weeks. The same volume of saline was used in the CON and CIH groups.

2.2. Histopathological examination

After carefully excising the heart, the ratios of heart weight/body weight (HW/BW) and left ventricle weight/body weight (LVW/BW) were used to estimate cardiac hypertrophy and left ventricular hypertrophy. Then, the isolated left ventricles were cleaned with PBS. Part of the myocardium of each heart was fixed in 4% paraformaldehyde for further histological study, and the rest of the myocardium was prepared as myocardial homogenate for assessment of markers of oxidative stress.

The hearts were removed from 4% paraformaldehyde, washed with tap water and dehydrated with serial dilutions of alcohol. Specimens were cleared in xylene and embedded in paraffin for 24 h. Paraffin-embedded tissue blocks were cut cross-sectionally into 4-μm-thick sections. The sections were stained with haematoxylin and counter-stained with eosin (H&E) following standard procedures. Masson's trichrome staining for fibrosis was also performed in heart sections. After mounting, the sections stained with H&E were imaged by a TissueFAXS viewer (TissueGnostics, Vienna, Austria), while the sections stained with Masson's trichrome were imaged using a DM3000 microscope (Leica, Solms, Germany). ImageJ was used to identify the degree of fibrosis.

2.3. Immunofluorescence

Heart slices were deparaffined and rehydrated. After permeabilization, an immunofluorescent assay was performed on the heart slices to

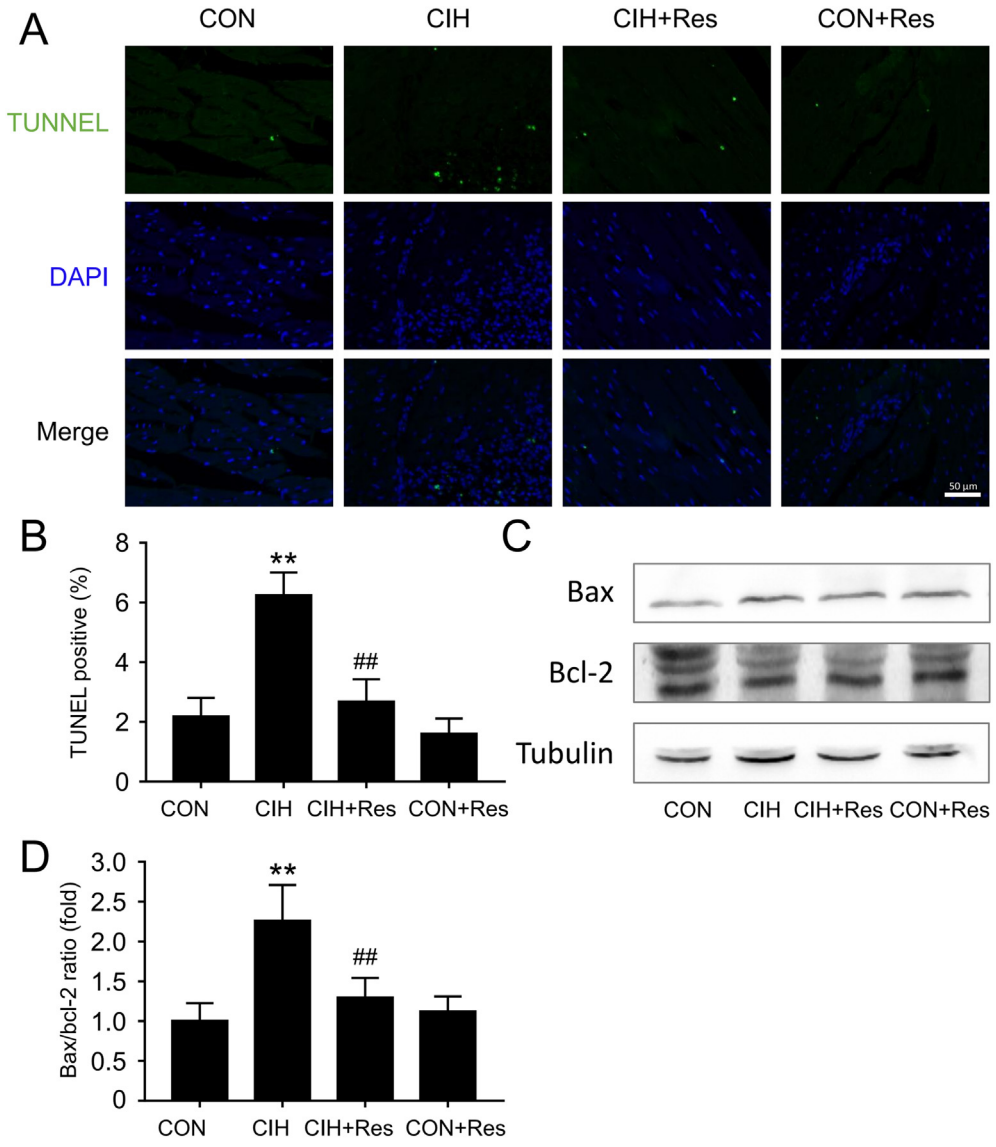

Fig. 3. Cell apoptosis in cardiac tissues. A. Representative images of TUNEL-stained sections. B. TUNEL-positive sections. C. Bax and bcl-2 expression levels determined by western blot. D. The bax/bcl-2 ratio was analysed. n = 6. $^{**}p < 0.01$ vs. control group; $^{\#\#}p < 0.01$ vs. CIH group.

examine myocardial cell hypertrophy by staining with FITC-labelled wheat germ agglutinin (WGA, 1:100, Invitrogen, Carlsbad, CA, USA), followed by incubation with DAPI. Photoshop (Adobe Photoshop 7.0, Adobe Systems Incorporated, San Jose, CA, USA) and ImageJ (ImageJ, National Institutes of Health, Bethesda, MD, USA) were used to identify the shortest diameter and cross-sectional area of a single myocardial cell, respectively.

Rabbit polyclonal anti-mTOR (phospho S2448) antibody (1:100, Abcam, USA) was used as the primary antibody to detect the p-mTOR content in the heart slices. Cy3-labelled anti-rabbit IgG (Zhongshan Golden Bridge Biotechnology, Beijing, China) was used to detect the primary antibody. Afterward, the slices were stained with 4′,6-diamidino-2-phenylindole (DAPI) and observed under a fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany). The level of p-mTOR expression was calculated using ImageJ.

2.4. Echocardiography

At the end of the experimental period, echocardiographic measurements of the left ventricular anterior wall in systole, left ventricular posterior wall in systole, ejection fraction (EF) and fractional shortening (FS) were determined by biplane echocardiography in the rats via an imaging system (Vevo 2100, VisualSonics Inc., Toronto, Canada) with an MS250 ultrasound transducer as described previously [13]. Briefly, the rats were anaesthetized, and their chests were shaved. M-mode recording of the short-axis view was performed on the shaved chest wall.

2.5. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

TUNEL was performed to identify apoptosis in the heart by a commercial kit (Roche Diagnostics GmbH, Mannheim, Germany). Briefly, the sections were covered with equilibration buffer at room temperature. Fifty microliters of TUNEL reaction mixture was blotted around the equilibrated areas for 60 min at 37 °C. After labelling the tissue sections with DAPI and mounting, images were taken under a fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany).

2.6. Oxidative stress assays

Malondialdehyde (MDA), superoxide dismutase (SOD), catalase

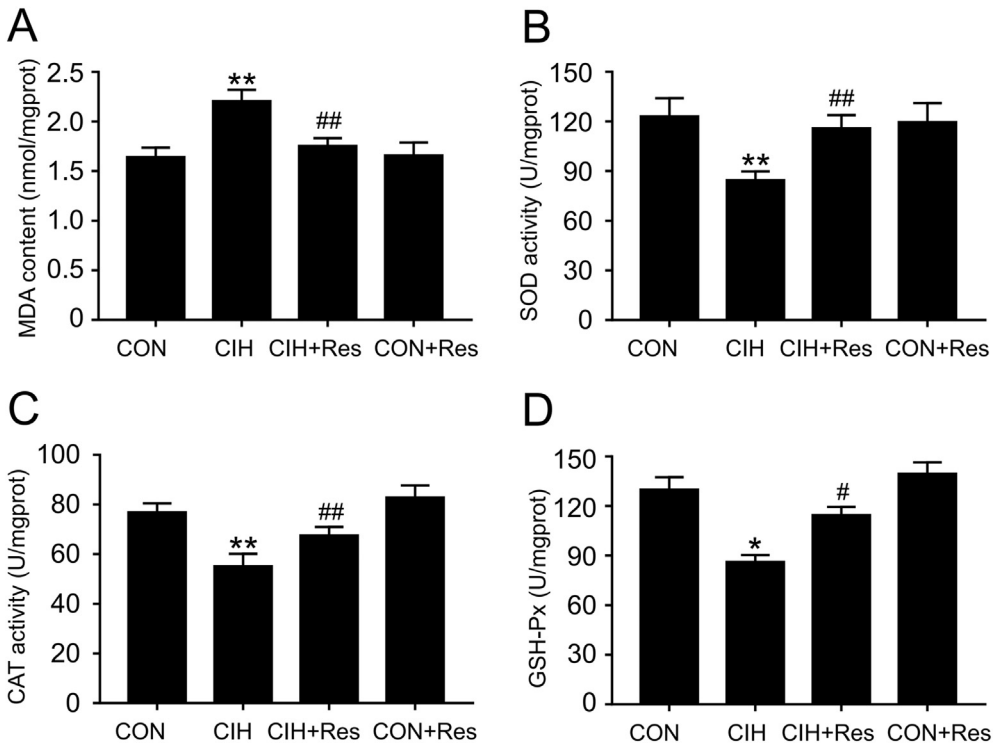

Fig. 4. Oxidative stress in cardiac tissues. A. MDA content. B. SOD activity. C. CAT activity. D. GSH-Px activity. n = 6. *$p < 0.05$ vs. control group; **$p < 0.01$ vs. control group; $^{\#}p < 0.05$ vs. CIH group; $^{\#\#}p < 0.01$ vs. CIH group.

(CAT) and glutathione peroxidase (GSH-Px) levels were determined in the heart homogenate by commercial kits (Nanjing Jiancheng Bioengineering Institute, China). Protein content was detected with a bicinchoninic acid protein assay kit (CWBio, Beijing, China). The MDA, SOD, CAT, and GSH-Px parameters were recorded on a microplate reader (Thermo Fisher, Waltham, MA, USA) at wavelengths of 550 nm, 550 nm, 405 nm, and 412 nm, respectively.

2.7. Western blotting

Myocardial tissues were homogenized with RIPA buffer (Thermo Fisher Scientific, USA) containing a protease inhibitor cocktail (R&D Systems Inc., Minneapolis, MN, USA). The extracted proteins were electrophoresed in SDS-PAGE and transferred onto polyvinylidene fluoride or polyvinylidene difluoride (PVDF) membranes (Millipore Co, Bedford, MA). The proteins were probed with the following primary antibodies: anti-bcl-2 (1:1000) from Immunoway; anti-tubulin (1:1000), anti-bax (1:1000), anti-LC3 I/II (1:1000), anti-beclin-1 (1:1000), anti-p62 (1:1000) and anti-mTOR (1:1000) from Servicebio; anti-AKT (1:1000), anti-p-AKT (1:3000), and anti-PI3K (1:500) from Proteintech; and anti-p-mTOR (1:1000) from Abcam. Tubulin was used as the internal control. After blocking, the membranes blotted for tubulin, bax, and p-AKT were incubated with a horseradish peroxidase-conjugated goat anti-mouse IgG (1:5000, Servicebio, Wuhan, China). Other membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG at a 1:3000 dilution (Servicebio, Wuhan, China). The signals were visualized by enhanced chemiluminescence detection (Vilber Fusion FX5 Spectra, Paris, France). Protein expression was represented as a fold change versus control.

2.8. Immunohistochemistry

Immunohistochemistry was employed to detect LC3 expression. Briefly, sections were deparaffinized and rehydrated, and heat-induced antigen retrieval was performed after blocking. The sections were treated with anti-LC3 rabbit IgG antibody (1:200, Servicebio, Wuhan, China) overnight, followed by goat anti-rabbit IgG-HRP (Zhongshan Biotechnology, Beijing, China). Finally, the chromogenic substrate 3,3′ diaminobenzidine (DAKO, Glostrup, Denmark) was used to visualize the positive immunocytochemical reaction. ImageJ software was used to measure the level of semi-quantitative LC3 expression by average optical density analysis.

2.9. Statistical analysis

analysed with Prism version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA) and presented as the mean ± standard error of the mean (SEM). Comparisons between groups were made using one-way analysis of variance (ANOVA) followed by a Bonferroni-Dunn test. A probability value $p < 0.05$ was considered significant.

3. Results

3.1. Resveratrol reverses CIH-induced myocardial hypertrophy

As shown in Fig. 1A, the width of the left ventricular wall showed a significant increase, while the ventricular chamber was smaller than that of the control group. Structure deteriorated improved by treatment with resveratrol. Exposure to CIH caused significant collagen deposition in the myocardial interstitial space, while deposition was obviously inhibited by treatment with resveratrol (Fig. 1B). The HW/BW ratio was significantly increased by CIH treatment ($p < 0.01$). Resveratrol significantly reversed this effect (Fig. 1C, $p < 0.05$). Compared with CIH-treated rats, rats treated with resveratrol showed a significant decrease in the LVW/BW ratio, indicating that resveratrol could alleviate CIH-induced left ventricular hypertrophy (Fig. 1D). Cell size quantification of WGA-stained sections with ImageJ was used to evaluate cardiac myocyte hypertrophy (Fig. 1E). We showed that both the shortest diameter (Fig. 1F, $p < 0.01$) and cross-sectional area (Fig. 1G, $p < 0.01$) of cardiac myocytes in the CIH group were higher compared

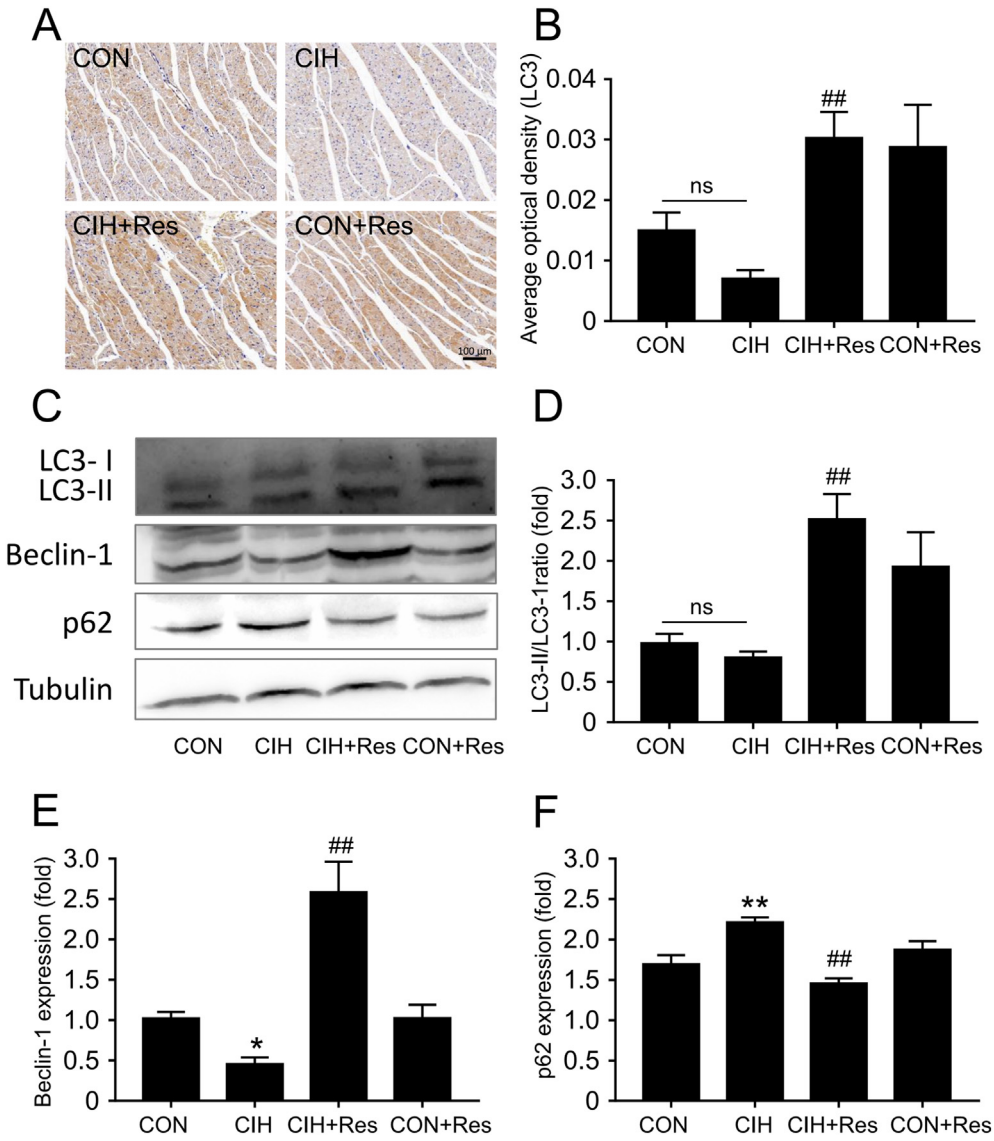

Fig. 5. The expression of LC3 I, LC3 II, p62 and beclin-1. A. Representative immunohistochemical images of LC3 staining. B. Semi-quantitative analysis of LC3 expression. C. Representative western blot images of LC3 I, LC3 II, p62 and beclin-1 levels. D. LC3 II/I ratio. E. Beclin-1 expression. F. p62 expression. n = 6. $^{*}p < 0.05$ vs. control group; $^{**}p < 0.01$ vs. control group; $^{\#\#}p < 0.01$ vs. CIH group; ns, not significant.

with those of control rats. These increases were largely ameliorated by resveratrol ($p < 0.01$ in the shortest diameter and $p < 0.05$ in the cross-sectional area).

3.2. Resveratrol reverses CIH-induced cardiac dysfunction

A representative M-mode parasternal short-axis view is presented in Fig. 2A. The LV anterior wall thickness in systole presented no noteworthy change among all the groups (Fig. 2B). Increased posterior wall thickness in systole was observed in CIH-treated rats, indicating that CIH could promote cardiac hypertrophy. Treatment with resveratrol improved ventricular remodelling as evidenced by the reduction in LV posterior wall thickness (Fig. 2C). Lower EF (Fig. 2D) and FS (Fig. 2E) values indicated cardiac systolic dysfunction in the CIH rat model. Resveratrol improved cardiac systolic function, as shown by increased EF and FS. Gastric perfusion of resveratrol alone resulted in no significant change in cardiac function compared with that of control rats.

3.3. Resveratrol inhibits CIH-induced myocardial apoptosis

TUNEL staining was performed to evaluate the inhibitory effect of resveratrol on CIH-induced cardiomyocyte apoptosis. The TUNEL positive index was higher in the CIH group than in the control group. Resveratrol obviously decreased apoptosis induced by CIH (Fig. 3A and B). The bax/bcl-2 ratio obtained from the western blot analysis revealed that apoptosis of cardiomyocytes was also increased in the CIH group ($p < 0.01$). The Bax/bcl-2 ratio decreased significantly after resveratrol administration (Fig. 3C and D, $p < 0.01$).

3.4. Resveratrol reduces CIH-induced heart oxidative stress

CIH-induced oxidative stress may be involved in the pathogenesis of myocardial hypertrophy. The effect of resveratrol treatment on the levels of oxidative stress markers is shown in Fig. 4. The data showed that the MDA level in the heart was significantly increased in the CIH group ($p < 0.01$). However, the presence of resveratrol significantly mitigated this increase (Fig. 4A, $p < 0.01$). SOD (Fig. 4B, $p < 0.01$), CAT (Fig. 4C, $p < 0.01$) and GSH-Px (Fig. 4D, $p < 0.05$) activities were

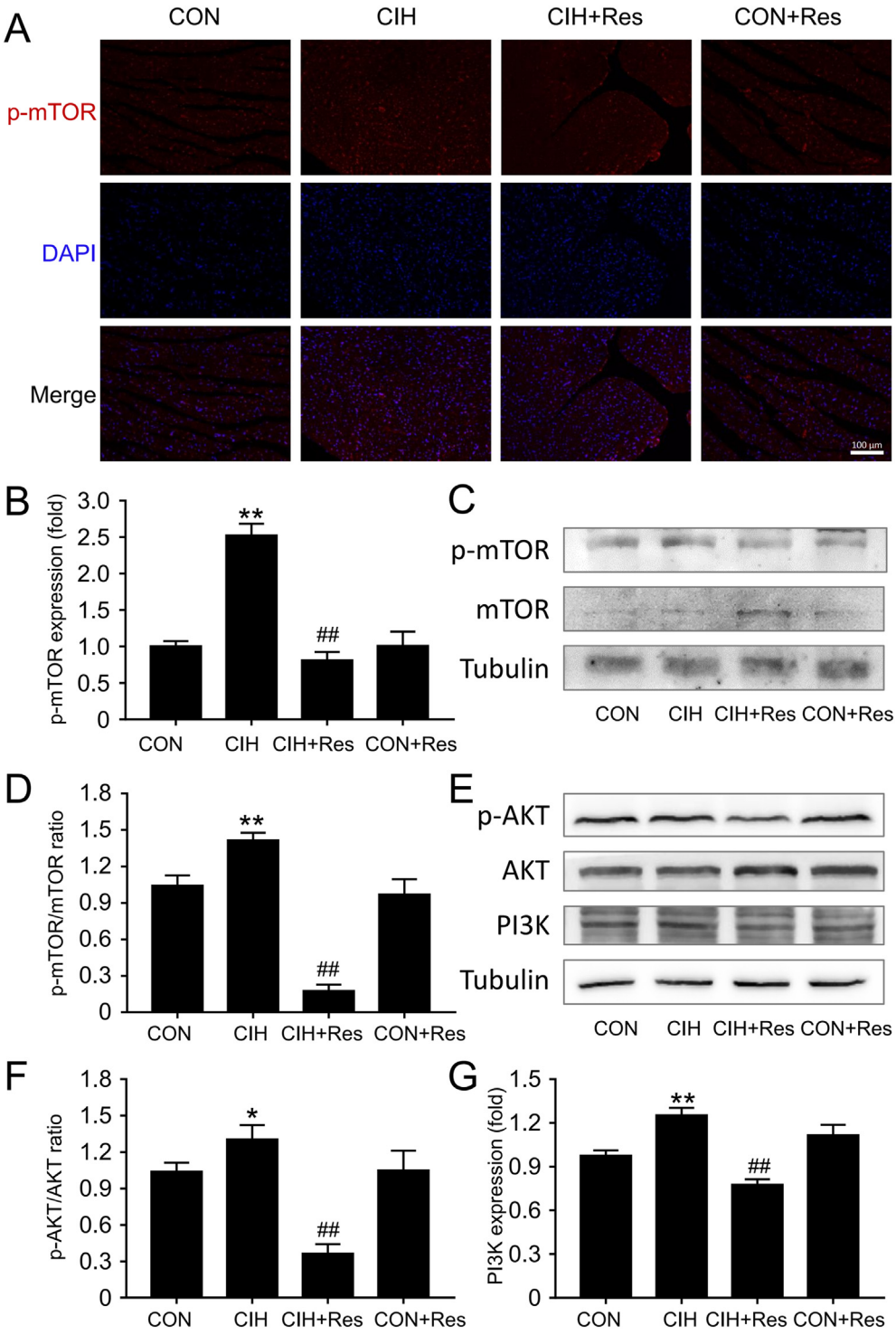

Fig. 6. The expression of mTOR, PI3K and AKT. A. Representative immunofluorescent images of p-mTOR staining. B. Semi-quantitative analysis of p-mTOR expression. C. Representative western blot images of p-mTOR and mTOR levels. D. p-mTOR/mTOR ratios. E. Representative western blot images of p-AKT, AKT and PI3K levels. F. p-AKT/AKT ratios. G. PI3K expression. n = 6. $^{*}p < 0.05$ vs. control group; $^{**}p < 0.01$ vs. control group; $^{\#\#}p < 0.01$ vs. CIH group.

decreased by CIH, when compared with control rats. Lipid peroxidation induced by CIH is counteracted by a free radical-scavenging mechanism, as shown by the increased SOD, CAT, and GSH-Px levels after resveratrol administration.

3.5. Resveratrol increases autophagy

The modulatory effect of resveratrol on autophagy in hearts of rats exposed to CIH was examined. LC3 staining experiments (Fig. 5A) indicated that LC3 expression was decreased in the CIH group, but the difference was not significant ($p = 0.1517$). In contrast, resveratrol caused a significant 3.32-fold increase in LC3 expression in the rats in the CIH group (Fig. 5B, $p < 0.01$). The levels of autophagy-related proteins (LC3, beclin-1, and p62) were assessed further by western blots (Fig. 5C). The data showed that there was no statistically significant difference in the ratio of LC3II/LC3I (Fig. 5D), and the expression of

beclin-1 was significantly decreased in the CIH group (Fig. 5E, $p < 0.05$) when compared with the control rats. However, the expression of these markers was significantly elevated after resveratrol administration (both $p < 0.01$). The results revealed that the autophagic flux was blocked by CIH as p62 expression showed a significant increase (Fig. 5F, $p < 0.01$). However, resveratrol caused a significant decrease in p62 expression compared with that in the CIH group.

3.6. Resveratrol attenuates CIH-induced PI3K/AKT/mTOR overactivation

It is generally accepted that hypertrophy is mediated by the phosphoinositide-3 kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signalling pathway [2]. According to the immunofluorescence staining for p-mTOR (Fig. 6A), p-mTOR expression was obviously increased in the CIH group ($p < 0.01$). However, that increase was significantly reversed after resveratrol administration (Fig. 6B, C and D, $p < 0.01$). Furthermore, the expression of p-AKT and PI3K was assessed by western blotting (Fig. 6E). The data revealed that the p-AKT/AKT ratio in a heart of CIH-treated rats was significantly higher than that of control rats (Fig. 6F, $p < 0.05$). Compared with that in the CIH group, the ratio of p-AKT/AKT in the CIH + Res group was significantly decreased ($p < 0.01$). Moreover, the expression of PI3K in the heart was significantly higher in the CIH group (Fig. 6G, $p < 0.01$), and PI3K expression was downregulated in the CIH + Res group ($p < 0.01$).

4. Discussion

Previous studies have demonstrated that OSA has various comorbidities. As a cardiovascular complication induced by repetitive obstructed breathing events, left ventricular cardiac hypertrophy has attracted increasing attention. We found that CIH exposure led to myocardial thickening and fibrosis. Moreover, both HW/BW and LVW/BW ratios were increased under CIH. In addition, the shortest diameter and cross-sectional area were significantly upregulated by CIH in cardiomyocytes. These results are in line with other animal studies on CIH-induced cardiac hypertrophy. Xie et al. reported that CIH exposure for six weeks significantly increased the size and changed the geometrical shape of the left ventricle in rats [35]. It was also reported by Imano et al. that IH for 28 days caused an increase in cardiomyocyte cross-sectional area and myofibre disarray [17]. A previous clinical study also revealed that the LV posterior wall was relatively thick in OSA patients [16]. The mechanism by which OSA induces cardiac hypertrophy is complex. One explanation is the increased afterload from the negative intrathoracic pressure generated by episodic apnoea [11]. Early cardiac overloading stimuli can provoke adaptive physiological cardiac hypertrophy with increased cardiac size and a normal and/or enhanced cardiac function [25]. Even though physiological cardiac hypertrophy can normalize wall tension, it would convert to pathological cardiac hypertrophy and progress to heart failure if the primary cause is sustained and quantitatively excessive [6]. In the current study, exposing rats to CIH for five weeks resulted in cardiac hypertrophy and dysfunction, which was partially ameliorated by resveratrol supplementation.

Apoptosis is considered a factor that links CIH with cardiac hypertrophy because apoptosis of cardiomyocytes can lead to increased workload on the remaining cells as cardiomyocytes are non-dividing [14]. Li et al. [19] identified that CIH induced increased expression of pro-apoptotic cytokines and their receptors in CIH-induced cardiac hypertrophy rats. Additionally, a cold-treated mouse trial showed that resveratrol effectively ameliorated cold exposure-induced cardiac hypertrophy and alleviated the damaged cardiac ultrastructure and function in mice at least partially by inhibiting cardiomyocyte apoptosis [38]. Some researchers have even proposed that cardiomyocyte apoptosis would promote the transition from compensated hypertrophy to heart failure [21]. It thus appears that apoptosis is a contributing factor for cardiac hypertrophy. Consistent with published results, we also found that CIH-treated rats showed a significant increase in TUNEL-positive cells and bax/bcl-2 ratio and that resveratrol treatment could prevent CIH-induced apoptosis.

Given the available evidence, oxidative stress resulting from reactive oxygen species can directly induce apoptosis [36]; consequently, oxidative stress markers in the cardiac homogenate were detected in the present study. CIH induced significantly higher MDA content than that in the control treatment. In addition, CIH decreased SOD, CAT, and GSH-Px activities in cardiac tissue homogenates. Furthermore, resveratrol administration resulted in an improved antioxidation capability. We inferred that increased propagation of oxidative stress plays a key role in CIH-mediated myocardiocyte apoptosis and cardiac dysfunction, while resveratrol may perform a beneficial role via the elimination of oxidative stress.

Autophagy is an important self-cannibalizing, degradative process in the pathogenesis of cardiac hypertrophy. However, the exact role of autophagy in cardiac hypertrophy remains controversial. Basic autophagy is acknowledged to be essential to maintaining cellular homeostasis by eliminating superfluous materials [37]. On the other hand, autophagy induced myocardiocyte degeneration and resulted in cardiac hypertrophy, suggesting that mitigating autophagy is beneficial to improve cardiac function [3]. However, decreased autophagy is reported to disturb energy and protein metabolism and induce severe cardiac hypertrophy [41]. Li et al. found that rapamycin could inhibit cardiac hypertrophy by restoring cardiac autophagy in miR-199a transgenic mice [22]. Our data also showed similar results. The current study indicated that CIH also induced impaired autophagy, while a high level of autophagy may improve heart dysfunction. Rapamycin has been found to protect against cardiac hypertrophy by restoring autophagy [31]. Administration of AMPK activators also mitigated transverse aortic constriction-induced cardiac hypertrophy in mice [24]. Blocking an autophagy-inhibiting miRNA increased autophagy and rescued pressure overload-induced cardiac hypertrophy [33]. The results of the present study revealed that resveratrol may improve heart function by restoring autophagy.

Several studies have suggested that, by increasing protein synthesis, mTOR is a positive regulator of cardiac hypertrophy [28]. Coincidentally, mTOR has also been identified as a negative regulator of autophagy. In this study, cardiac hypertrophy was alleviated by resveratrol in rats exposed to CIH, and autophagy was restored. Inhibition of mTOR by resveratrol might play a role in the process. We also demonstrated that CIH activates PI3K and AKT, a signalling pathway associated with mTOR activity. Downregulation of PI3K-interacting protein 1 by siRNA increased myocardiocyte hypertrophy through the activation of the PI3K/AKT/mTOR pathway [32]. AKT is acknowledged as an inducer of cardiac hypertrophy. AKT knock-down mice showed alleviated cardiac hypertrophy induced by exercise [5]. Considering the possible regulation of the PI3K/AKT/mTOR signalling pathway on cardiac hypertrophy, negative regulation of the pathway may improve cardiac hypertrophy. Resveratrol treatment inhibited the PI3K/AKT/mTOR signalling pathway and restored autophagy levels in a heart of CIH-exposed rats, exhibiting protective effects against cardiac hypertrophy.

Overall, our study demonstrated that CIH impaired myocardiocyte autophagy and led to apoptosis, which resulted in the induction of cardiac hypertrophy. Resveratrol could alleviate cardiac hypertrophy and dysfunction by restoring autophagy and inhibiting apoptosis.

Acknowledgment

This work was supported by National Natural Science Foundation of China (Grant No. 81170069) (ESJ), Science and Technology Research Fund Project of Hebei Colleges and Universities (Grant No. ZD2016087), Graduate Innovation Fund of Hebei University of Chinese Medicine (Grant No. XCXZZBS2019001).

Declaration of competing interest

The authors have no conflicts of interest to disclose.

References

[1] A. Abdullah, G. Eigbire, A. Salama, A. Wahab, N. Nadkarni, R. Alweis, Relation of obstructive sleep apnea to risk of hospitalization in patients with heart failure and preserved ejection fraction from the National Inpatient Sample, Am. J. Cardiol. 122 (2018) 612–615.

[2] T. Aoyagi, T. Matsui, Phosphoinositide-3 kinase signaling in cardiac hypertrophy and heart failure, Curr. Pharm. Des. 17 (2011) 1818–1824.

[3] I.P. Chou, Y.P. Chiu, S.T. Ding, B.H. Liu, Y.Y. Lin, C.Y. Chen, Adiponectin receptor 1 overexpression reduces lipid accumulation and hypertrophy in the heart of diet-induced obese mice–possible involvement of oxidative stress and autophagy, Endocr. Res. 39 (2014) 173–179.

[4] F. da Silva Paulitsch, L. Zhang, Continuous positive airway pressure for adults with obstructive sleep apnea and cardiovascular disease: a meta-analysis of randomized trials, Sleep Med. 54 (2019) 28–34.

[5] B. DeBosch, I. Treskov, T.S. Lupu, C. Weinheimer, A. Kovacs, M. Courtois, et al., Akt1 is required for physiological cardiac growth, Circulation 113 (2006) 2097–2104.

[6] Dorn GW, 2nd. The fuzzy logic of physiological cardiac hypertrophy. Hypertension. 2007;49:962–70.

[7] J. Fan, X. Wang, X. Ma, V.K. Somers, S. Nie, Y. Wei, Association of Obstructive Sleep Apnea with Cardiovascular Outcomes in patients with acute coronary syndrome, J. Am. Heart Assoc. 8 (2019) e010826.

[8] J.S. Floras, Sleep apnea and cardiovascular disease: an enigmatic risk factor, Circ. Res. 122 (2018) 1741–1764.

[9] N. Fourny, C. Lan, E. Seree, M. Bernard, M. Desrois, Protective effect of resveratrol against ischemia-reperfusion injury via enhanced high energy compounds and eNOS-SIRT1 expression in type 2 diabetic female rat heart, Nutrients 11 (2019).

[10] C.M. Goes, P. Falcochio, L.F. Drager, Strategies to manage obstructive sleep apnea to decrease the burden of atrial fibrillation, Expert. Rev. Cardiovasc. Ther. 16 (2018) 707–713.

[11] C.A. Goudis, D.G. Ketikoglou, Obstructive sleep and atrial fibrillation: pathophysiological mechanisms and therapeutic implications, Int. J. Cardiol. 230 (2017) 293–300.

[12] J. Gu, Y.Q. Fan, H.L. Zhang, J.A. Pan, J.Y. Yu, J.F. Zhang, et al., Resveratrol suppresses doxorubicin-induced cardiotoxicity by disrupting E2F1 mediated autophagy inhibition and apoptosis promotion, Biochem. Pharmacol. 150 (2018) 202–213.

[13] P. Guan, X.M. Lin, S.C. Yang, Y.J. Guo, W.Y. Li, Y.S. Zhao, et al., Hydrogen gas reduces chronic intermittent hypoxia-induced hypertension by inhibiting sympathetic nerve activity and increasing vasodilator responses via the antioxidation, J. Cell. Biochem. 120 (2019) 3998–4008.

[14] T. Hang, Z. Huang, S. Jiang, J. Gong, C. Wang, D. Xie, et al., Apoptosis in pressure overload-induced cardiac hypertrophy is mediated, in part, by adenine nucleotide translocator-1, Ann. Clin. Lab. Sci. 36 (2006) 88–95.

[15] M. Hasan, H. Bae, An overview of stress-induced resveratrol synthesis in grapes: perspectives for resveratrol-enriched grape products, Molecules 22 (2017).

[16] J. Hedner, H. Ejnell, K. Caidahl, Left ventricular hypertrophy independent of hypertension in patients with obstructive sleep apnoea, J. Hypertens. 8 (1990) 941–946.

[17] H. Imano, R. Kato, S. Tanikawa, F. Yoshimura, A. Nomura, Y. Ijiri, et al., Factor Xa inhibition by rivaroxaban attenuates cardiac remodeling due to intermittent hypoxia, J. Pharmacol. Sci. 137 (2018) 274–282.

[18] A. Kuno, R. Hosoda, R. Sebori, T. Hayashi, H. Sakuragi, M. Tanabe, et al., Resveratrol ameliorates Mitophagy disturbance and improves cardiac pathophysiology of dystrophin-deficient mdx mice, Sci. Rep. 8 (2018) 15555.

[19] G. Li, M. Jin, Y. He, J. Ren, M. Zhang, Y. Chen, et al., Fork head box class O1 (FOXO1) activates Bim expression to mediate cardiac apoptosis in chronic intermittent hypoxia-induced cardiac hypertrophy, Med. Sci. Monit. 23 (2017) 3603–3616.

[20] K. Li, Z. Chen, Y. Qin, Y.X. Wei, Plasm YKL-40 levels are associated with hypertension in patients with obstructive sleep apnea, Biomed. Res. Int. 2019 (2019) 5193597.

[21] Z. Li, O.H. Bing, X. Long, K.G. Robinson, E.G. Lakatta, Increased cardiomyocyte apoptosis during the transition to heart failure in the spontaneously hypertensive rat, Am. J. Phys. 272 (1997) H2313–H2319.

[22] Z. Li, Y. Song, L. Liu, N. Hou, X. An, D. Zhan, et al., miR-199a impairs autophagy and induces cardiac hypertrophy through mTOR activation, Cell Death Differ. 24 (2017) 1205–1213.

[23] Z. Li, J. Wang, X. Yang, Functions of autophagy in pathological cardiac hypertrophy, Int. J. Biol. Sci. 11 (2015) 672–678.

[24] A. Nakai, O. Yamaguchi, T. Takeda, Y. Higuchi, S. Hikoso, M. Taniike, et al., The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress, Nat. Med. 13 (2007) 619–624.

[25] J.Y. Ooi, B.C. Bernardo, J.R. McMullen, The therapeutic potential of miRNAs regulated in settings of physiological cardiac hypertrophy, Future Med. Chem. 6 (2014) 205–222.

[26] A. Qaseem, J.E. Holty, D.K. Owens, P. Dallas, M. Starkey, P. Shekelle, et al., Management of obstructive sleep apnea in adults: a clinical practice guideline from the American College of Physicians, Ann. Intern. Med. 159 (2013) 471–483.

[27] A.A. Sabe, N.Y. Elmadhun, R.S. Dalal, M.P. Robich, F.W. Sellke, Resveratrol regulates autophagy signaling in chronically ischemic myocardium, J. Thorac. Cardiovasc. Surg. 147 (2014) 792–798 (Discussion 8-9).

[28] S. Sciarretta, M. Forte, G. Frati, J. Sadoshima, New insights into the role of mTOR signaling in the cardiovascular system, Circ. Res. 122 (2018) 489–505.

[29] H. Sekizuka, N. Osada, Y.J. Akashi, Impact of obstructive sleep apnea and hypertension on left ventricular hypertrophy in Japanese patients, Hypertens. Res. 40 (2017) 477–482.

[30] K. Shinmura, K. Tamaki, M. Sano, M. Murata, H. Yamakawa, H. Ishida, et al., Impact of long-term caloric restriction on cardiac senescence: caloric restriction ameliorates cardiac diastolic dysfunction associated with aging, J. Mol. Cell. Cardiol. 50 (2011) 117–127.

[31] W. Soesanto, H.Y. Lin, E. Hu, S. Lefler, S.E. Litwin, S. Sena, et al., Mammalian target of rapamycin is a critical regulator of cardiac hypertrophy in spontaneously hypertensive rats, Hypertension 54 (2009) 1321–1327.

[32] H.K. Song, J. Kim, J.S. Lee, K.J. Nho, H.C. Jeong, J. Kim, et al., Pik3ip1 modulates cardiac hypertrophy by inhibiting PI3K pathway, PLoS One 10 (2015) e0122251.

[33] A. Ucar, S.K. Gupta, J. Fiedler, E. Erikci, M. Kardasinski, S. Batkai, et al., The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy, Nat. Commun. 3 (2012) 1078.

[34] E. Vizzardi, E. Sciatti, I. Bonadei, A. D'Aloia, A. Curnis, M. Metra, Obstructive sleep apnoea-hypopnoea and arrhythmias: new updates, J. Cardiovasc. Med. (Hagerstown) 18 (2017) 490–500.

[35] S. Xie, Y. Deng, Y.Y. Pan, Z.H. Wang, J. Ren, X.L. Guo, et al., Melatonin protects against chronic intermittent hypoxia-induced cardiac hypertrophy by modulating autophagy through the 5′ adenosine monophosphate-activated protein kinase pathway, Biochem. Biophys. Res. Commun. 464 (2015) 975–981.

[36] H. Yang, Y. Xie, D. Yang, D. Ren, Oxidative stress-induced apoptosis in granulosa cells involves JNK, p53 and puma, Oncotarget 8 (2017) 25310–25322.

[37] Z. Yang, D.J. Klionsky, Eaten alive: a history of macroautophagy, Nat. Cell Biol. 12 (2010) 814–822.

[38] K. Yin, L. Zhao, D. Feng, W. Ma, Y. Liu, Y. Wang, et al., Resveratrol attenuated low ambient temperature-induced myocardial hypertrophy via inhibiting cardiomyocyte apoptosis, Cell. Physiol. Biochem. 35 (2015) 2451–2462.

[39] J.S. You, G.B. Anderson, M.S. Dooley, T.A. Hornberger, The role of mTOR signaling in the regulation of protein synthesis and muscle mass during immobilization in mice, Dis. Model. Mech. 8 (2015) 1059–1069.

[40] T. Young, L. Finn, P.E. Peppard, M. Szklo-Coxe, D. Austin, F.J. Nieto, et al., Sleep disordered breathing and mortality: eighteen-year follow-up of the Wisconsin sleep cohort, Sleep 31 (2008) 1071–1078.

[41] T. Zaglia, G. Milan, A. Ruhs, M. Franzoso, E. Bertaggia, N. Pianca, et al., Atrogin-1 deficiency promotes cardiomyopathy and premature death via impaired autophagy, J. Clin. Invest. 124 (2014) 2410–2424.