Mechanistic analysis of resveratrol in cardiac hypertrophy by network pharmacology and animal experiments

SHAN REN[1*], LEI SHEN[2*], SONG LIN[3], DAN XIAO[4], WEI XIAO[1], PEI-MEI YAN[1], YAN-YAN ZHANG[1], WEI-WEI JIA[3] and YAN LIN[1]

[1]Department of Pathophysiology, [2]Department of Anatomy, [3]Research Center and [4]Department of Psychiatry, Qiqihar Medical University, Qiqihar, Heilongjiang 161006, P.R. China

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Abstract. Resveratrol (Res) serves a protective role in hepatic, cardiovascular and autoimmune hypertrophic disease. However, the mechanisms by which Res ameliorates cardiac hypertrophy have not yet been fully elucidated. In the present study, network pharmacology was used to construct a network and perform enrichment analysis to evaluate the effect of Res on cardiac hypertrophy. Experimental validation was performed using 40 Sprague-Dawley rats administered intragastric 80 mg/kg/day Res and 20 mg/kg/day 3-methyladenine (3-MA) for 4 weeks. A total of 444 targets associated with cardiac hypertrophy and 229 potential disease-associated targets of Res were identified, from which 8 overlapping genes were demonstrated. Gene Ontology function and 'Kyoto Encyclopedia of Genes and Genomes' pathway enrichment analysis demonstrated that Res affected STAT3 and was associated with autophagy signaling pathways, including 'negative regulation of autophagy for hypertrophic cardiomyopathy'. Furthermore, Res ameliorated isoprenaline-induced cardiac hypertrophy, significantly improving cardiac dysfunction *in vivo* experiment (echocardiography, the degree of ventricular hypertrophy, etc.); this effect may be associated with regulation of autophagy and apoptosis. The autophagy inhibitor 3-MA markedly reversed the anti-cardiac hypertrophy effects of Res. In conclusion, Res inhibited cardiac hypertrophy via downregulation of the apoptosis signaling pathway and upregulating the autophagy pathway.

Correspondence to: Professor Yan Lin, Department of Pathophysiology, Qiqihar Medical University, 333 Bukui Street, Qiqihar, Heilongjiang 161006, P.R. China
E-mail: yanlinqqhr@aliyun.com

*Contributed equally

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Introduction

Myocardial hypertrophy is an independent risk factor for cardiovascular disease and a key cause of heart failure (1). The condition may be caused by valvular disease, hypertension, myocardial infarction and cardiomyopathy amongst other pathological mechanisms (1). Cardiomyocyte hypertrophy is an indicator of myocardial remodeling and induces decreased myocardial contractility, which progresses to heart failure (2). Therefore, it is necessary to determine factors offering a protective effect against cardiac hypertrophy to decrease the incidence of heart failure and subsequent mortality. Resveratrol (Res) is a natural non-flavonoid polyphenol with anti-inflammatory, cardioprotective, neuroprotective, antioxidant and anti-aging properties that is abundant in food such as grapes, mulberries and blueberries (3,4). Preclinical studies have reported the potential of Res as a treatment for severe cardiomyopathy (5,6). However, mechanisms underlying the cardioprotective action of Res remain unknown.

Network pharmacology is a powerful multi-component, multi-target tool which has been used to study therapeutic mechanisms of herbal medicines (7,8). The approach has been employed in mechanistic studies associated with numerous types of chronic disease, including obesity, diabetes, chronic obstructive pulmonary and cardio-cerebral vascular disease (9,10). Therefore, evaluation of the mechanistic actions of multi-target drugs in the treatment of complex diseases draws on abundant datasets to inform preliminary investigation.

The present study evaluated the pharmacological action of Res in cardiac hypertrophy using network pharmacology and animal experiments. Potential Res targets were predicted and cross-referenced with therapeutic targets associated with cardiomyopathy. Res targets associated with cardiac hypertrophy were integrated to establish a multi-target network. Cytoscape and Metascape were used to analyze significant genes in the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. Significant targets and pathways obtained from network pharmacology were verified using western blotting. The process of network pharmacology and animal experiments is presented in Fig. 1.

Materials and methods

Construction of Res target library. The chemical structure of Res was downloaded from Pubchem (pubchem.ncbi.nlm.nih.gov) and Res targets were predicted and downloaded from the Traditional Chinese Medicine Systems (TCMSP; https://tcmsp-e.com/) and Swiss Target Prediction (swisstargetprediction.ch) databases (the default parameters of the websites were utilized), using the inclusion criteria of P≤0.5 (11). Genes corresponding to targets were identified from UniProt (12).

Acquisition of cardiac hypertrophy gene dataset. Target genes associated with cardiac hypertrophy were downloaded from the Gene Expression Omnibus database (GEO; ncbi.nlm.nih.gov/geo). The gene expression profiles of myocardial tissue removed surgically from 54 male and 52 female patients with hypertrophic cardiomyopathy were downloaded from the GSE36961 dataset (P<0.05; $\log_2$ fold change >1).

Molecular docking and enrichment analysis. The structure of the signal transducer and activator of transcription (STAT3) protein was downloaded from the Protein Data Bank (rcsb.org/) and molecular docking performed using AutoDock Vina (version, 1.2.0) (13). The online Database for Annotation, Visualization and Integrated Discovery (david.ncifcrf.gov/home.jsp) enrichment analysis service was used to analyze enriched genes using GO terms and KEGG pathways. Venn diagrams were generated using Venny 2.1.0 (bioinfogp.cnb.csic.es/tools/venny/) to demonstrate potential associations between datasets. Functional GO and KEGG analysis of potential targets of Res for cardiac hypertrophy treatment was performed using Metascape (version, 3.5) (metascape.org) and pathways with P≤0.05 were considered to be statistically significant for cardiac hypertrophy treatment. A diagram of Res-associated signaling pathways based on GO and KEGG analyses was generated using the 'Pathview' package in the Toolkit for Biologists software (version, 1.098667) (14).

Protein-protein interaction (PPI) network. Interactions between potential Res targets associated with hypertrophic cardiomyopathy in both males and females were evaluated using a PPI network generated using the BisoGenet 1.0 plugin (apps.cytoscape.org/apps/bisogenet) for Cytoscape 3.9 (15). The Molecular Complex Detection (MCODE) plugin (version 2.0.2, apps.cytoscape.org/apps/mcode) for Cytoscape 3.9 was used to visualize protein-protein interactions with topology parameters evaluated using the Cytoscape network analyzer tool. Compound-target associations were presented in a pharmacological network map constructed using Cytoscape 3.9 (the default parameters were utilized). Core gene-encoding proteins were screened for using MCODE score >5.

Animal experiments. A total of 20 healthy adult female and 20 male Sprague Dawley rats (eight weeks of age, 200-250 g) were supplied by the Animal Center of Qiqihar Medical University. Rats were housed with temperature and humidity at 22±2˚C and 45±15%, respectively, under a 12 h light-dark cycle, with free access to food and water. Rats were acclimated for 7 days prior to the experiment. Rats were randomly divided into four groups (each group contained 5 male and 5 female rats) as follows: i) Control; ii) isoprenaline (ISO); iii) Res and iv) Res + 3-Methyladenine (3-MA). Rats in the ISO group were administered 0.9% saline by gavage (in equal volume to Res group, about 2 ml) for 4 weeks and subcutaneous ISO injection (5 mg/kg/day) on days 22-28. The Res group were administered Res (80 mg/kg/day) by gavage for 4 weeks (16) and subcutaneous ISO injections (5 mg/kg/day) from day 22-28. The Res + 3-MA group was administered Res (80 mg/kg/day) by gavage, 3-MA (20 mg/kg/day) by intraperitoneal injection for 4 weeks and subcutaneous ISO injection (5 mg/kg/day) on days 22-28. Rats in the control group were administered 0.9% saline (in equal volume to Res group, about 2 ml) by gavage for 4 weeks and subcutaneous 0.9% saline (in equal volume to ISO, about 0.5 ml) injections on days 22-28. Rats were euthanized using intraperitoneal injection of 3% sodium pentobarbitone (240 mg/kg) following echocardiography and the ventricular myocardia was excised. The present study was approved by the Committee on the Ethics of Animal Experiments of Qiqihar Medical University (Qiqihar, heilingjiang, approval no. QMU-AECC-2019-53).

Echocardiography. Echocardiography was performed 24 h after the final treatment. Briefly, rats were anaesthetized with intraperitoneal injection of 3% sodium pentobarbitone (80 mg/kg) and the thoracic area was shaved. M-mode recording of the short-axis view was performed on the shaved chest wall. Left ventricular end-systolic dimension (LVESd), LV end-diastolic dimension (LVEDd), LV fractional shortening (FS) and ejection fraction (EF) were assessed by echocardiography using a V6 imaging system [VINNO Technology (Suzhou) Co., Inc.] with high-frequency ultrasound transducer.

Tissue collection and analysis. The heart and left ventricle of euthanized rats were isolated. The ratio of left ventricle weight:body weight (LVW/BW) and heart weight:BW (HW/BW) were calculated to evaluate the degree of ventricular hypertrophy.

Measurement of malondialdehyde (MDA) and lactate dehydrogenase (LDH). MDA and LDH levels in heart homogenate was assessed using MDA assay kit and LDH assay kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol and optical density at 530 and 450 nm was quantified using a microplate reader (Thermo Fisher Scientific, Inc.).

Measurement of atrial natriuretic peptide (ANP) mRNA expression levels. Total RNA was isolated from left ventricle tissue with TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) .According to the manufacturer's protocols, total RNA was reverse transcribed with mRNA reverse transcription kit (cat. no. RR037A, Takara Bio, Inc.).The resulting cDNA was used as template for RT-qPCR using SYBR-Green Master Mix (cat. no. A25742, Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions, the PCR program was as follows: 95˚C for 30 sec, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. Melting curves were used to ensure that only the correct product was amplified. Primer sequences were as follows: ANP forward (F), 5'-GGG

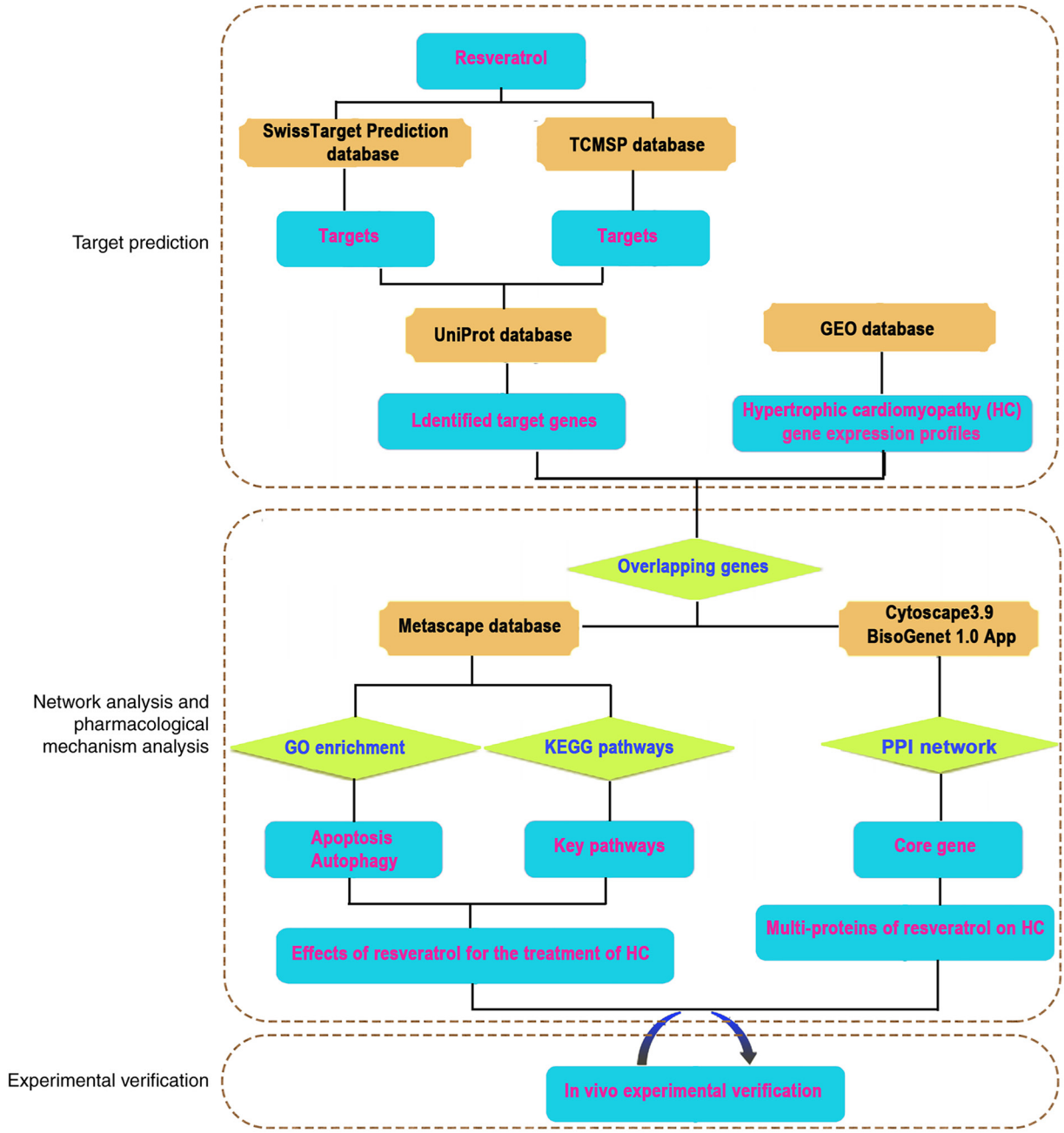

Figure 1. Flowchart of our research. TCMSP, traditional Chinese medicine systems pharmacology database and analysis platform; GEO, Gene Expression Omnibus; HC, hypertrophic cardiomyopathy; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction.

AAGTCAACCCGTCTCA-3' and reverse (R), 5'-GGGCTC CAATCCTGTCAAT-3' and GAPDH F, 5'-GAGACAGCC GCATCTTCTTG-3' and R, 5'-ATACGGCCAAATCCG TTCAC-3'. Data were normalized to expression of GAPDH mRNA as endogenous control and quantified using the $2^{-\Delta\Delta Cq}$ method (17).

Western blotting. Myocardial tissue was homogenized with cold RIPA buffer with 1% phenylmethylsulfonyl fluoride (P0013C, Beyotime Institute of Biotechnology), Total protein concentrations were measured by a BCA kit (Beyotime Institute of Biotechnology). Equal amounts of protein (30 μg) was separated using 8 or 10% SDS-PAGE and electro-transferred to PVDF membranes (MilliporeSigma). Membranes were blocked with 5% blocking buffer for 1 h at room temperature and incubated with primary antibodies as follows: Anti-ANP (1:1,000; cat. no. sc-515701, Santa Cruz Biotechnology, Inc.), anti-Bcl-2 (1:1,000; cat. no. sc-7382, Santa Cruz Biotechnology, Inc.), anti-Bax (1:1,000; cat. no. sc-20067, Santa Cruz Biotechnology, Inc.), anti-cytochrome C (CytC; 1:1,000; cat. no. sc-13156, Santa Cruz Biotechnology, Inc.) and anti-autophagy-related gene 5 (Atg5; 1:1,000; cat. no. sc-133158, Santa Cruz Biotechnology, Inc.) at 4˚C overnight. GAPDH (1:1,000; sc-166545, Santa Cruz Biotechnology, Inc) was used as the internal control. Membranes were incubated with Secondary goat antimouse antibody were used (diluted 1:5,000, cat. no. BA1050; Boster Biological Technology, Ltd.) for 1.5 h at room temperature. The membranes were washed

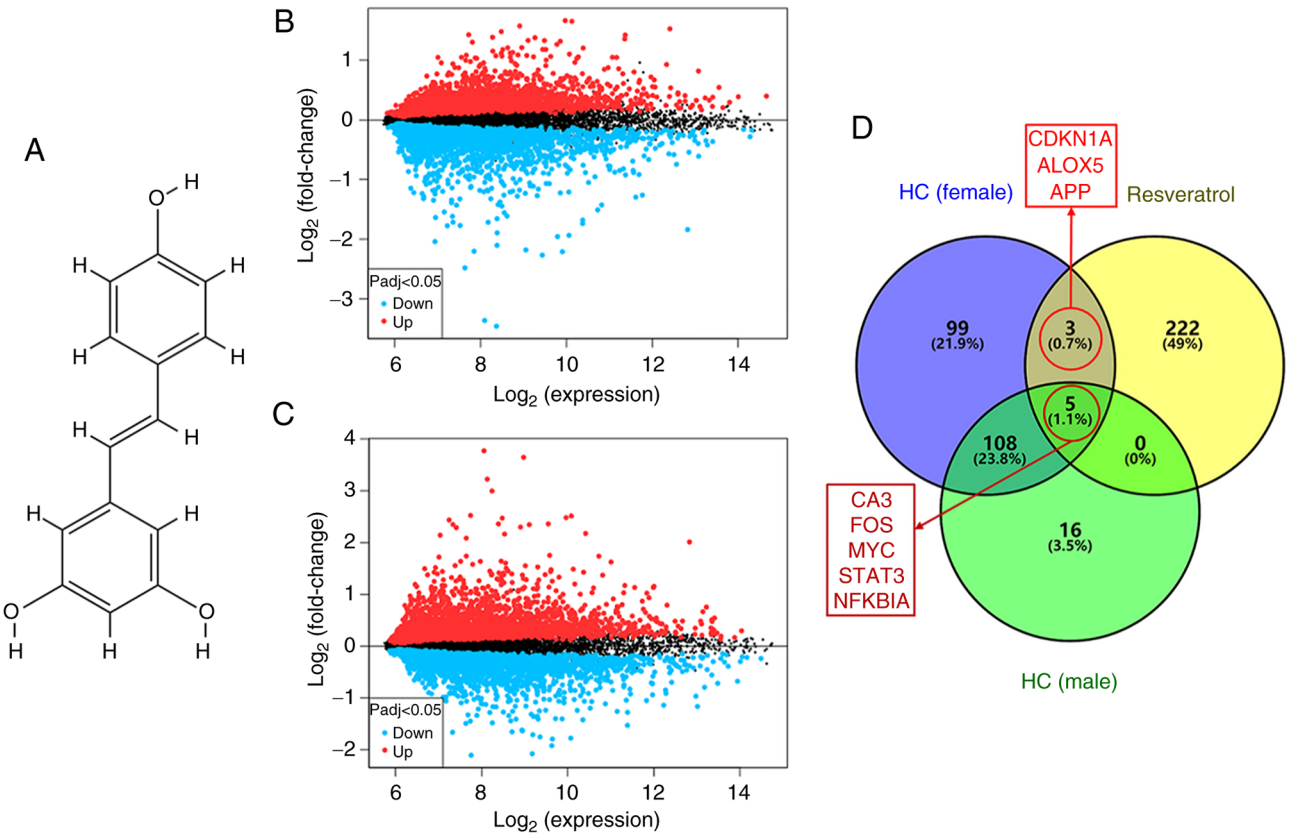

Figure 2. Target characteristics of resveratrol for HC. (A) Molecular structure of resveratrol. (B) A total of 129 differential gene expression profiles for male HC, demonstrating 43 up- and 86 downregulated genes. (C) A total of 215 differential gene expression profiles in female HC, demonstrating 140 up- and 75 downregulated genes. (D) Common targets of resveratrol and HC illustrated using a Venn diagram. HC, hypertrophic cardiomyopathy; CDKN1A, cyclin-dependent kinase inhibitor 1A; ALOX5, arachidonate 5-lipoxygenase; APP, amyloid β precursor protein; CA3, carbonic anhydrase 3; FOS, Fos proto-oncogene; NFKBIA, NFκB inhibitor α.

three times in Wash Buffer and proteins were detected using ECL reagent (Beyotime Institute of Biotechnology). Relative protein expression levels were semi-quantified using ImageJ software (National Institutes of Health; version 1.48) and presented as fold-change compared with control.

Statistical analysis. All data are presented as mean ± SEM and represent at least three independent experiments. Statistical comparisons were made one-way ANOVA followed by a post hoc analysis (Tukey test) and GraphPad Prism software (GraphPad Software, Inc.,version 7.0). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Res target characteristics for hypertrophic cardiomyopathy. The molecular structure of Res ($C_{14}H_{12}O_3$) is presented in Fig. 2A. A total of 230 potential Res targets from the TCMSP and Swiss Target Prediction databases and 444 cardiac hypertrophy-associated targets from the GSE36961 dataset, in the form of differential gene expression profiles for male and female patients with hypertrophic cardiomyopathy, were screened to characterizing the potential association between Res and cardiac hypertrophy (Fig. 2B and C). A total of 8 proteins overlapped in the intersection of Res and cardiac hypertrophy-associated targets, of which 3 were identified in females only (Fig. 2D).

Bioinformatics analysis of target genes. Molecular docking analysis demonstrated the binding of Res to STAT3 (Fig. 3). Enrichment analysis demonstrated a further 10 pathways, including 'IL-4 and IL-13 signaling' and 'TGF-β signaling pathway', that were associated with Res treatment of cardiac hypertrophy. Further analysis demonstrated that Res intervening pathways, including 'IL-5 signaling pathway' and 'lipid metabolism in senescent cells' were associated with 'apoptosis', whereas 'TGF-β signaling pathway' and 'positive regulation of glycolytic process' were associated with 'autophagy'. Therefore, apoptosis and autophagy may be potential therapeutic mechanistic targets of Res in hypertrophic cardiomyopathy.

PPI network of target genes. The BisoGenet 1.0 plugin for Cytoscape 3.9 was used to analyze the 129 Res targets associated with male patients with hypertrophic cardiomyopathy and a network of PPI associations with 1,206 nodes and 21,105 connections was constructed (Fig. 4A). A total of four modules of the core gene-encoded proteins had an MCODE score >5 as predicted using the MCODE plugin (version 2.0.2) in Cytoscape 3.9 (Fig. 4B). Analysis of the 215 Res targets associated with female patients with hypertrophic cardiomyopathy generated a PPI network with 3,350 nodes and 63,783 connections (Fig. 4C). A total of eight modules of the core gene-encoded proteins had an MCODE score >5, predicted

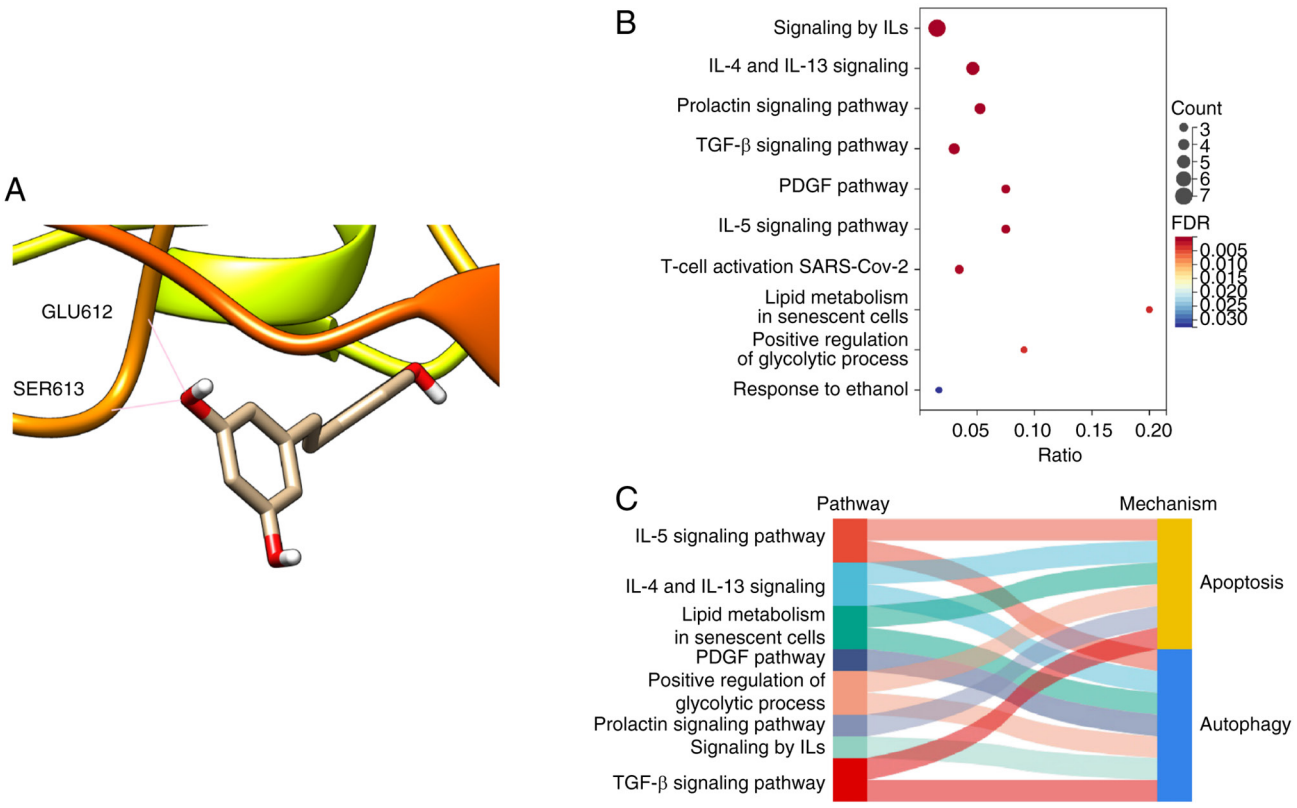

Figure 3. Molecular docking model and biological functions of targets. (A) Molecular docking model and interaction residues of the binding of Res to STAT3 (docking score, -4.9; interaction residues, Glu612 and Ser613). (B) Enrichment analysis of 8 overlapping targets. (C) Association of pathways regulated by Res with apoptosis and/or autophagy. Res, resveratrol; PDGF, platelet-derived growth factor; SARS, severe acute respiratory syndrome; FDR, false discovery rate.

using the MCODE plugin (version 2.0.2) in Cytoscape 3.9 (Fig. 4D). The female PPI network was more complex than the male network and featured more core proteins.

Res reverses ISO-induced myocardial hypertrophy. The cardiac index and ANP mRNA and protein expression levels were assessed as cardiac hypertrophy indicators. HW/BW, LVW/BW, the levels of ANP mRNA and protein expression were increased in ISO group. Treatment with Res significantly decreased HW/BW and LVW/BW values and decreased the levels of ANP mRNA and protein expression compared with the ISO group. Moreover, Res + 3-MA significantly increased the levels of ANP mRNA and protein expression compared with Res-alone (Fig. 5A-D).

Res reverses ISO-induced cardiac dysfunction. Echocardiographic parameters were assessed in M-mode (Fig. 6A); treatment with Res produced significant increases in EF% and FS% compared with the ISO group (Fig. 6B and C). These results demonstrated the importance of Res in cardiac hypertrophy. Furthermore, both LVESd and LVEDd were significantly decreased in the Res group compared with the ISO group. No significant differences were demonstrated for EF%, FS% and LVEDd between the Res and Res + 3-MA groups; however, LVESd exhibited a significant increase in the Res + 3-MA group compared with the Res group (Fig. 6D and E). In summary, ISO and 3-MA treatment both led to progressive cardiac enlargement, however, Res improved cardiac function.

Res decreases ISO-induced oxidative stress in the heart. The effect of Res on oxidative stress was investigated. Treatment with ISO significantly increased cardiac MDA and LDH levels compared with the control and these increases were significantly reversed by treatment with Res (Fig. 7). However, levels of MDA (Fig. 7A) were markedly higher and levels of LDH (Fig. 7B) significantly higher in the Res + 3-MA compared with the Res group.

Res downregulates apoptosis and upregulates autophagy. The protein expression levels of Bcl-2 and Atg5 were significantly decreased by ISO treatment compared with the control. Res caused significant upregulation of Bcl-2 and Atg5 expression compared with the ISO group. Expression levels of pro-apoptosis proteins Bax and CytC significantly increased in the ISO group compared with control. However, this effect was significantly decreased by Res treatment (Fig. 8A-E). However, Res + 3-MA treatment significantly reversed changes in protein expression levels of CytC, Bcl-2 and Atg5 compared with the Res group (Fig. 8A-E).

Discussion

Cardiac hypertrophy is an adaptive response to pathological stimuli but prolonged hypertrophy results in cardiac dysfunction and heart failure. Volume overload or elevated pressure is a key cause and pathological remodeling is characterized by hypertrophied cardiomyocytes, interstitial fibrosis, perivascular fibrosis and decreased cardiac compliance leading

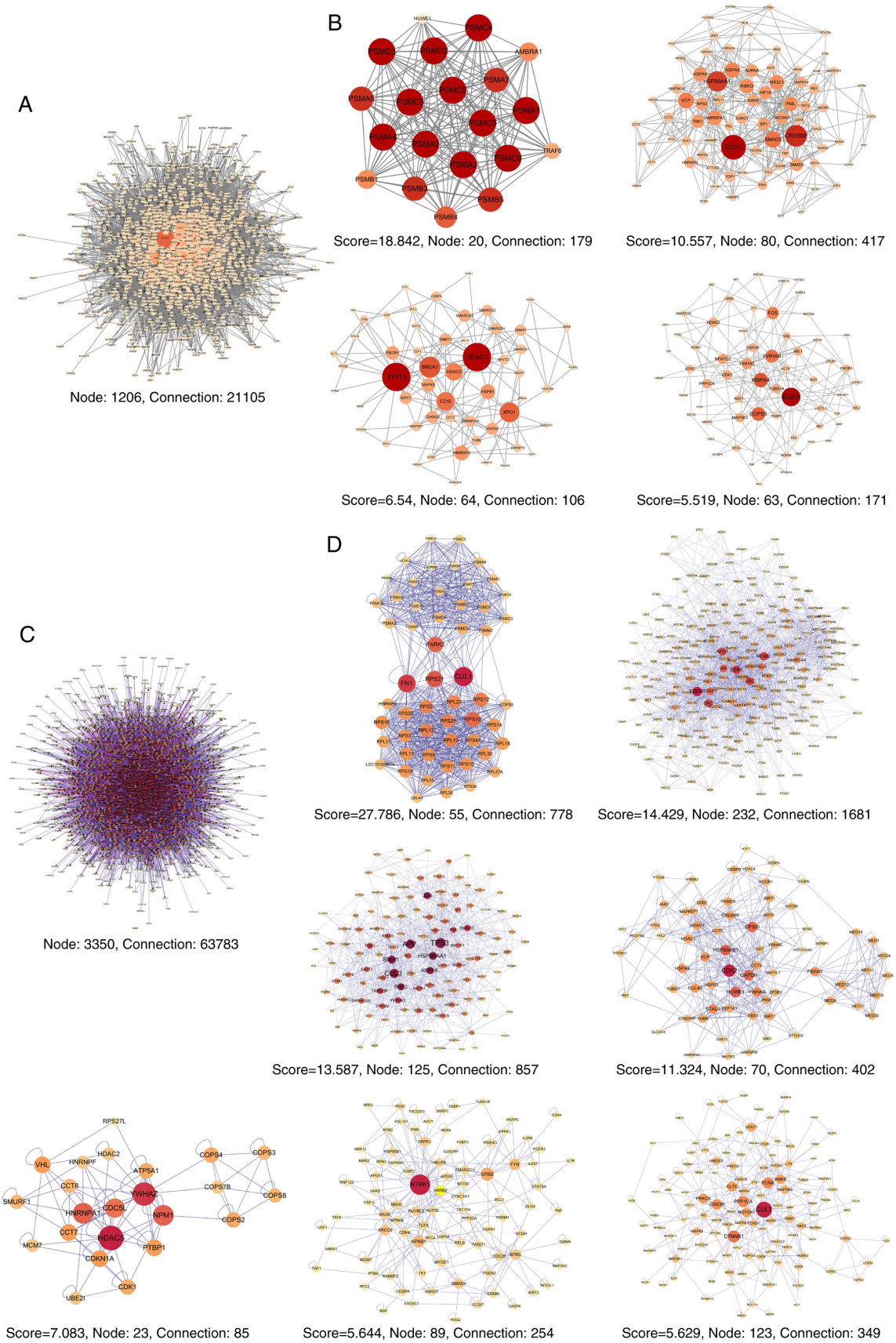

Figure 4. Network of protein-protein interactions of Res on hypertrophic cardiomyopathy with screening of core proteins. (A) Protein interaction network of Res in male patients with hypertrophic cardiomyopathy generated using the BisoGenet 1.0 plugin for Cytoscape 3.9, with node area and font size positively associated with protein degree value. (B) With regard to the effect of Res in male patients with HC, proteins encoded by core genes were screened using the CytoScape 3.9 MCODE plugin, with node area, font size and color intensity positively associated with the core protein degree values. (C) Protein interaction network of Res in female patients with hypertrophic cardiomyopathy generated using the BisoGenet 1.0 plugin for Cytoscape 3.9, with node area and font size positively correlated with protein degree value. (D) With regard to the effect of Res in female patients with HC, proteins encoded by core genes were screened using the CytoScape 3.9 MCODE plugin, with node area, font size and color intensity positively associated with the core protein degree values. Res, resveratrol.

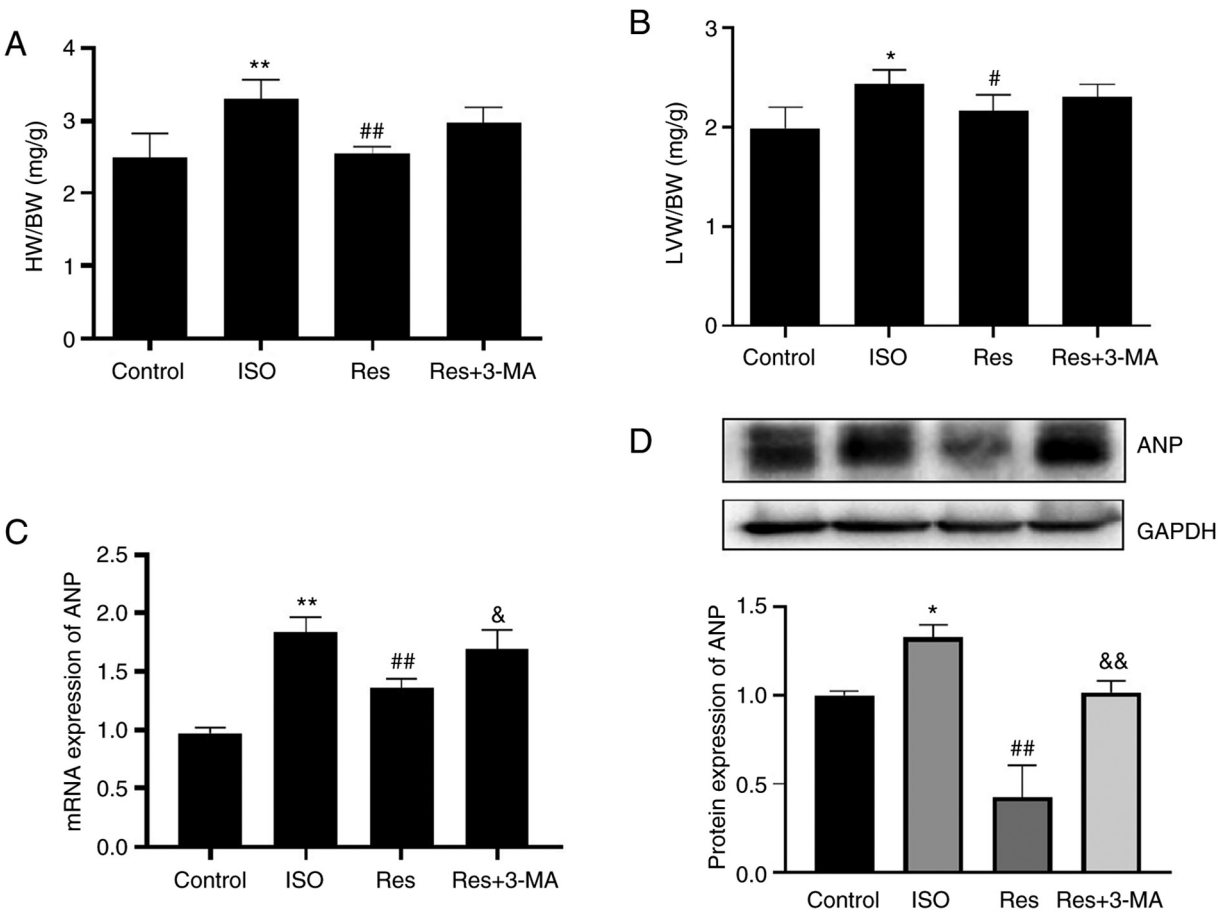

Figure 5. Res reverses ISO-induced myocardial hypertrophy. (A) HW/BW and (B) LVW/BW ratio. (C) mRNA expression levels of ANP were detected using reverse transcription-quantitative PCR. (D) Protein expression levels of ANP were detected using western blotting and normalized to GAPDH. *P<0.05 and **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. ISO; $^{\&}$P<0.05 and $^{\&\&}$P<0.01 vs. Res. ISO, isoprenaline; Res, resveratrol; 3-MA, 3-methyladenine; HW, heart weight; BW, body weight; LVW, left ventricle weight; ANP, atrial natriuretic peptide.

to malignant arrhythmia, heart failure and potential sudden cardiac death (18-20). A previous study demonstrated associations between cardiac hypertrophy and apoptosis, oxidative stress, autophagy and aging (21). These connections highlight the importance of elucidating the precise mechanism of cardiac hypertrophy.

Res is a plant-derived polyphenol present in grape skin, peanut, cranberries and veratrum (22). Numerous biological activities have been ascribed to Res, including potent anti-inflammatory, antioxidant, anti-aging, insulin sensitization and cardioprotective activities, which are reported to protect of nerve and heart tissue (16). Furthermore, anti-aging activity has been suggested due to upregulation of hydrogen peroxide-dependent autophagy (23). Res has also been reported to inhibit vascular smooth muscle remodeling and growth and proliferation of cardiac fibroblasts (24). Res (80 mg/kg) via intragastric administration has been reported to protect against L-arginine-induced ANP, which may be associated with enhancement of sirtuin 1-mediated deacetylation of p53 and heat shock transcription factor 1 (16). To the best of our knowledge, however, little detailed information regarding Res protective mechanisms has been reported.

Network pharmacology has allowed previous studies of the anti-cardiac hypertrophy mechanism of Res to be performed (25-27) and may facilitate identification of potential drug treatments for complex disease (28,29). The present study demonstrated the presence of 230 Res targets in the Swiss Target Prediction and TCMSP databases and 444 sex-specific targets associated with cardiac hypertrophy in the GSE36961 dataset. A total of 8 Res targets were also associated with cardiac hypertrophy, 5 in males and 8 in females. The intersection of Res and cardiac hypertrophy targets was visualized using the 'Res-target-disease' network and GO functional and KEGG pathway enrichment analyses were performed. STAT3, Myc and apoptotic processes were keytargets of Res in hypertrophic cardiomyopathy. Molecular docking analysis demonstrated the potential regulatory effect of Res on STAT3, a protein which is known to inhibit apoptosis and numerous autophagy-associated proteins (30). STAT3 is reported to be a suppressor of autophagy, which suggested that the Res therapeutic mechanism may involve autophagy in both male and female patients. Moreover, recent evidence suggests that STAT3 may promote cardiomyocyte metabolism and function by an effect on endothelial autophagy (31). The effect of STAT3 on autophagy has also been reported in cardiac fibrosis (32-34). In summary, effects of Res on apoptosis and autophagy may underlie the potential mechanisms of Res in treating cardiac hypertrophy.

PPI networks demonstrated that Res action in female patients with hypertrophic cardiomyopathy was more complex and contained more core proteins than the

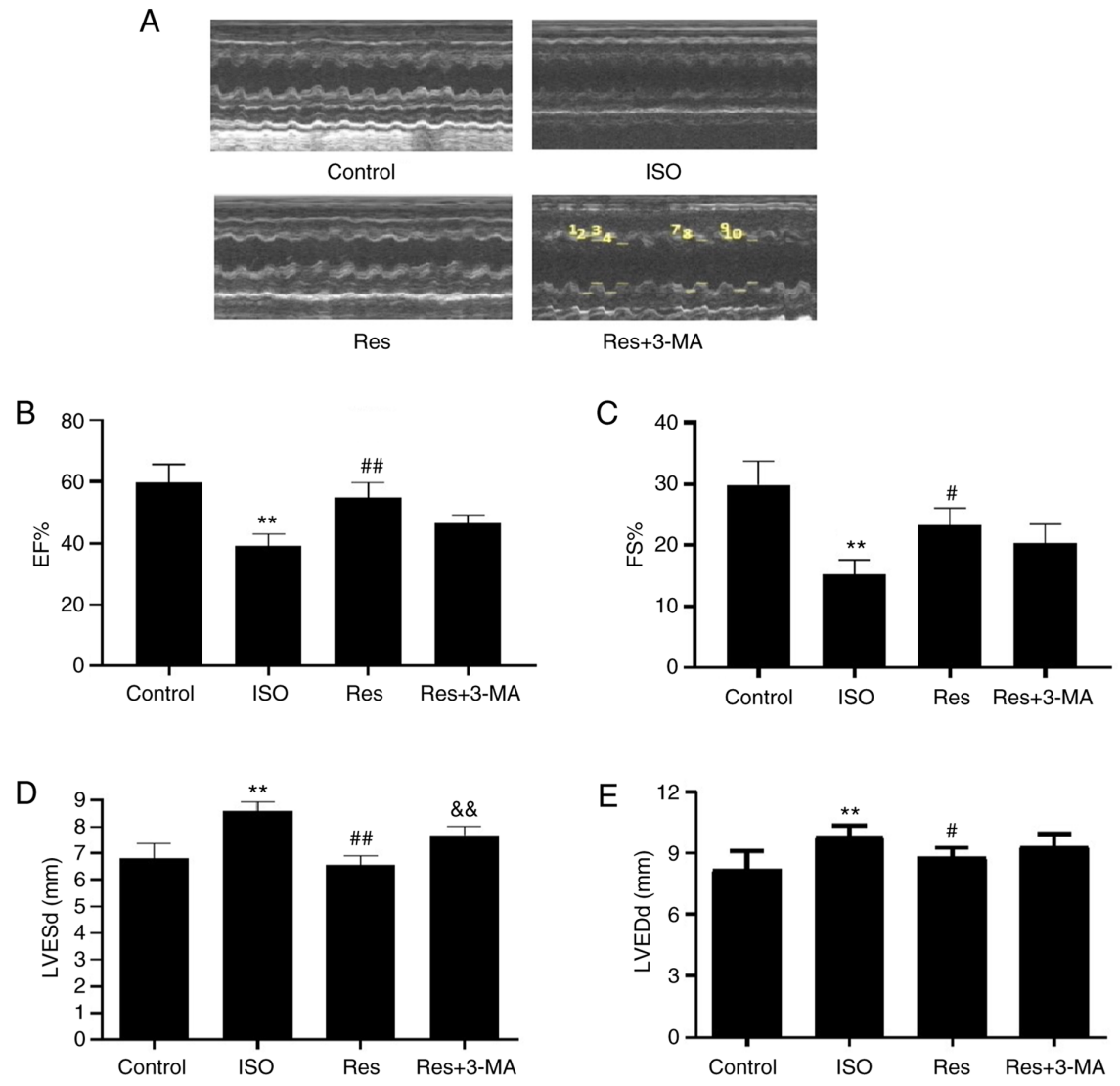

Figure 6. Res reverses ISO-induced cardiac dysfunction. Echocardiographic images of the left ventricle by the short-axis view and electrocardiography findings. (A) Representative M-mode echocardiograms in different groups. (B) EF and (C) FS were decreased in ISO group and were increased in Res group. (D) LVESd and (E) LVEDd were increased in ISO group and were decreased in Res group and LVESd was increased in Res+3-MA group. **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. ISO; $^{\&\&}$P<0.01 vs. Res. EF, ejection fraction; FS, fractional shortening; LVESd, left ventricular end systolic diameter; LVEDd, left ventricular end diastolic diameter; ISO, isoprenaline; Res, resveratrol; 3-MA, 3-methyladenine.

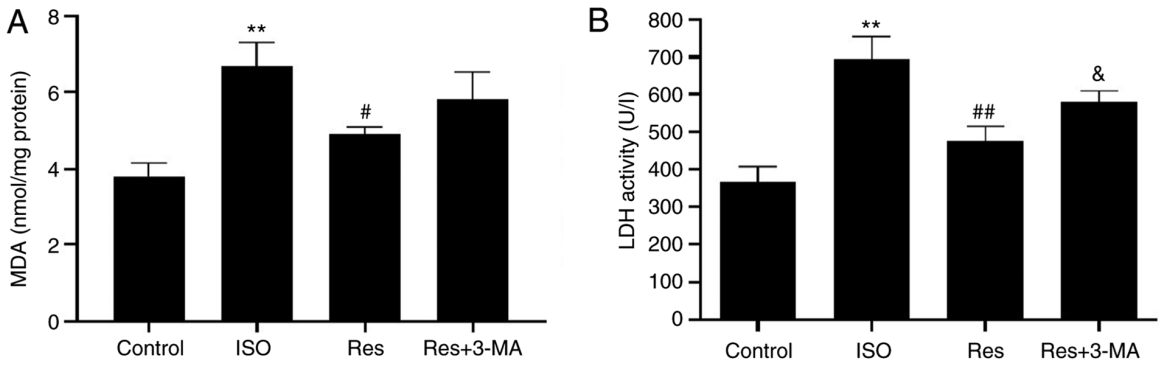

Figure 7. Res decreases ISO-induced oxidative stress of heart. (A) MDA and (B) LDH levels in heart tissues. **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. ISO; $^{\&}$P<0.05 vs. Res. MDA, malondialdehyde; LDH, lactate dehydrogenase; ISO, isoprenaline; Res, resveratrol; 3-MA, 3-methyladenine.

equivalent action in male patients with hypertrophic cardiomyopathy. In both physiological and pathological scenarios and pre-menopausal, females exhibit enhanced protection against cardiac hypertrophy compared with males. Such

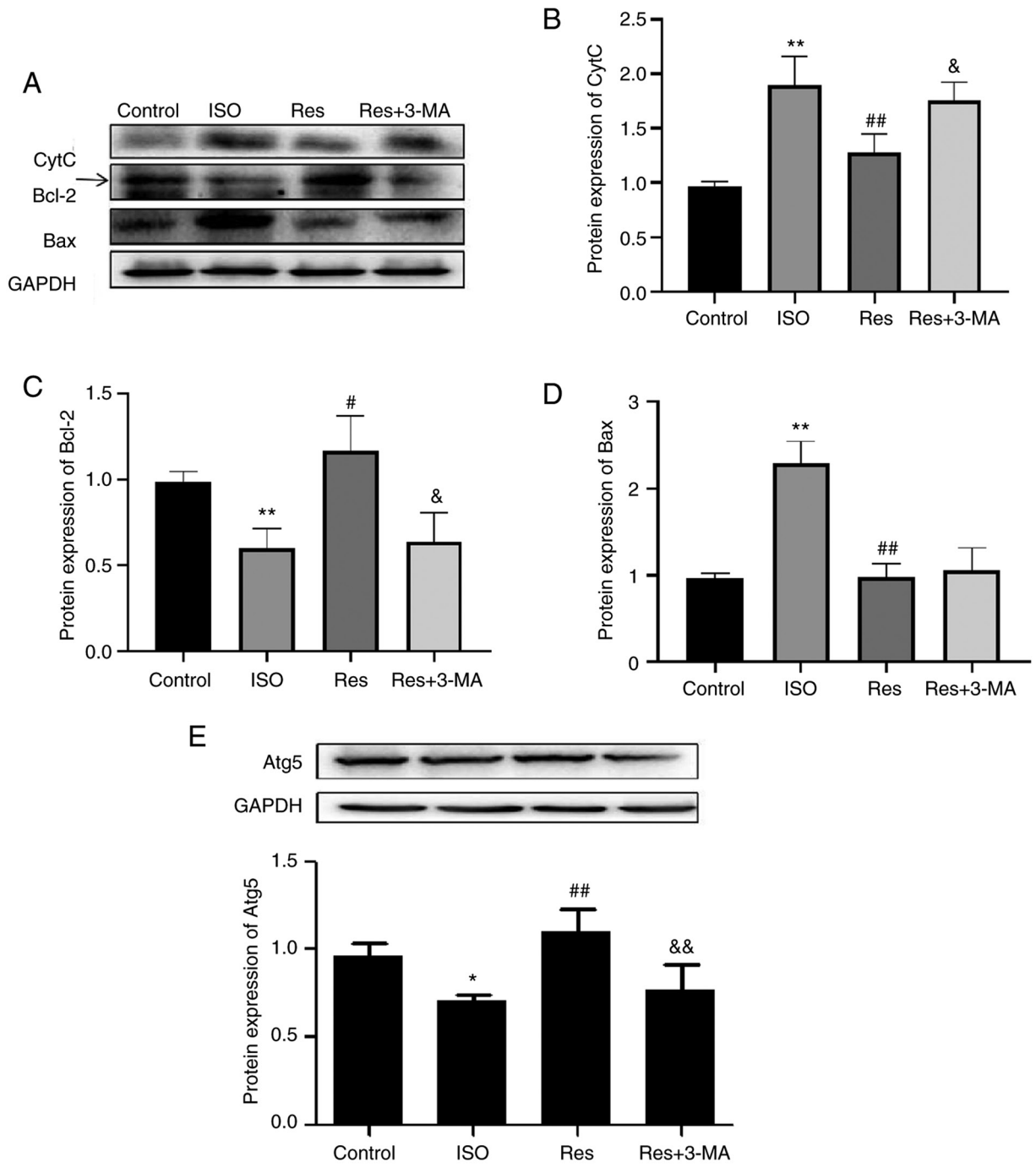

Figure 8. Res downregulates the apoptosis pathway and upregulates the autophagy pathway. (A) Protein expression levels of CytC, Bcl-2 and Bax. Semi-quantification of the protein expression levels of (B) CytC, (C) Bcl-2 (arrow) and (D) Bax normalized to GAPDH protein expression. (E) Protein expression levels of Atg5. *P<0.05 and **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. ISO; $^{\&}$P<0.05 and $^{\&\&}$P<0.01 vs. Res. CytC, cytochrome C; ISO, isoprenaline; Res, resveratrol; 3-MA, 3-methyladenine; Atg5, autophagy-related gene 5.

protection disappears following the menopause; however it can be partially restored by estrogen replacement therapy. Estrogen antagonizes the pro-hypertrophy signaling pathway, whereas androgens have the opposing effect (35). The network pharmacology approach, encompassing various pharmacological effects of traditional drugs and the interaction between chemical molecules and target proteins at the molecular level, demonstrates interactions which support the suggestion that Res achieves its cardioprotective role in inhibiting cardiomyocyte apoptosis by increasing autophagy (36).

Pathological indicators, such as ANP, MDA and LDH, cardiac morphological indicators, such as HW/BW and LVW/BW, and cardiac structural indicators, such as EF%, FS%, LVESd and LVEDd, were analyzed *in vivo* to demonstrate the effects of Res. Res is demonstrated to reverse cardiac hypertrophy via complex mechanisms which remain to be clarified; however autophagy appears to be a key factor (37). However, the role of autophagy in myocardial hypertrophy is controversial. Previous studies have reported activation of general autophagy as having both protective and detrimental roles during cardiac hypertrophy and heart failure (38,39).

Both Li *et al* (40) and Wang *et al* (41) reported that ISO-induced cardiac hypertrophy is accompanied by a significant decrease in autophagy and that enhanced autophagy may attenuate cardiac hypertrophy. The network pharmacology in the present study identified autophagy as a potential therapeutic Res target in cardiac hypertrophy. Furthermore, hypertrophic responses induced by ISO were alleviated by Res treatment via regulation of autophagy in the present study. Moreover, the autophagy inhibitor 3-MA stimulated hypertrophic responses in rats. However, HW/BW and LVW/BW, which demonstrated the therapeutic effect of Res on cardiac function, were not affected by 3-MA. However, 3-MA markedly decreased the reversal of Res-induced cardiac dysfunction, which suggested that the regulation of autophagy was involved. Apoptosis was also associated with the therapeutic effect of Res. Autophagy is reported to be associated with apoptosis, which affects homeostasis of the intracellular environment, initiated by both exogenous and endogenous pathways (42,43). The results of the present study demonstrated that apoptosis significantly increased following ISO treatment; this effect was significantly reversed by Res treatment. It could be hypothesized that 3-MA decreased the therapeutic effect of Res by suppressing autophagy and promoting apoptosis, two forms of programmed cell death. Autophagy may be regarded as a double-edged sword (44). Interactions between apoptosis and autophagy are highly complex and environmentally dependent and it can be concluded that both are mechanisms involved in the treatment of cardiac hypertrophy by Ras (45). Mitochondrial cytochrome C release and apoptosis are both inhibited by Bcl-2 binding to Bax (46). Bax is a proapoptotic member of the Bcl-2 family activated by apoptotic signaling, leading to cytochrome C release (47). This is stimulated by proapoptotic Bax, Bad, BH3-interacting domain death agonist and Bcl-2 antagonist/killer and inhibited by anti-apoptotic Bcl-xl and/or Bcl-2 (48). Res significantly reversed ISO-induced changes to Bcl-2, Bax and cytochrome C expression in the present study. 3-MA markedly reversed the regulation of apoptosis-associated proteins by Res, indicating that protection against myocardial hypertrophy involves autophagy and apoptosis. One potential limitation of this study would be that the mechanism of Res promoting the Atg5 require further study. Further investigation is therefore needed to understand the association between autophagy and the cardiac function following treatment with Res.

In conclusion, the results of the integrated network pharmacological and animal experiments in the present study demonstrated that Res ameliorated myocardial hypertrophy via regulation of autophagy and apoptosis-associated signaling pathways. These results may inform further studies on the use of Res in cardiac disease. Res may be an effective multi-target anti-myocardial hypertrophy drug.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SR, LS and YL designed the experiments and wrote and revised the manuscript. WWJ, SL and WX performed the experiments and analyzed the data. PMY, YYZ and DX performed the experiments.SR and LS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The experimental protocols were approved by the Committee on the Ethics of Animal Experiments of Qiqihar Medical University (Qiqihar, heilingjiang, approval no. QMU-AECC-2019-53).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Nakamura M and Sadoshima J: Mechanisms of physiological and pathological cardiac hypertrophy. Nat Rev Cardiol 15: 387-407, 2018.
2. Ott C, Jung T, Brix S, John C, Betz IR, Foryst-Ludwig A, Deubel S, Kuebler WM, Grune T, Kintscher U and Grune J: Hypertrophy-reduced autophagy causes cardiac dysfunction by directly impacting cardiomyocyte contractility. Cells 10: 805, 2021.
3. Shaito A, Posadino AM, Younes N, Hasan H, Halabi S, Alhababi D, Al-Mohannadi A, Abdel-Rahman WM, Eid AH, Nasrallah GK and Pintus G: Potential adverse effects of resveratrol: A literature review. Int J Mol Sci 21: 2084, 2020.
4. Tian B and Liu J: Resveratrol: A review of plant sources, synthesis, stability, modification and food application. J Sci Food Agric 100: 1392-1404, 2020.
5. Peñalver P, Belmonte-Reche E, Adán N, Caro M, Mateos-Martín ML, Delgado M, González-Rey E and Morales JC: Alkylated resveratrol prodrugs and metabolites as potential therapeutics for neurodegenerative diseases. Eur J Med Chem 146: 123-138, 2018.
6. Chen C, Zou LX, Lin QY, Yan X, Bi HL, Xie X, Wang S, Wang QS, Zhang YL and Li HH: Resveratrol as a new inhibitor of immunoproteasome prevents PTEN degradation and attenuates cardiac hypertrophy after pressure overload. Redox Biol 20: 390-401, 2019.
7. Wang Y, Yang SH, Zhong K, Jiang T, Zhang M, Kwan HY and Su T: Network pharmacology-based strategy for the investigation of the anti-obesity effects of an ethanolic extract of zanthoxylum bungeanum maxim. Front Pharmacol 11: 572387, 2020.
8. Lin X, Shao T, Huang L, Wen X, Wang M, Wen C and He Z: Simiao decoction alleviates gouty arthritis by modulating proinflammatory cytokines and the gut ecosystem. Front Pharmacol 11: 955, 2020.
9. Wang W, Liu T, Yang L, Ma Y, Dou F, Shi L, Wen A and Ding Y: Study on the multi-targets mechanism of triphala on cardio-cerebral vascular diseases based on network pharmacology. Biomed Pharmacother 116: 108994, 2019.

10. Wang J, Peng L, Jin L, Fu H and Shou Q: Network pharmacology analysis of the identification of phytochemicals and therapeutic mechanisms of paeoniae radix alba for the treatment of asthma. J Immunol Res 2021: 9659304, 2021.
11. Daina A, Michielin O and Zoete V: SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res 47 (W1): W357-W364, 2019.
12. UniProt Consortium: UniProt: The universal protein knowledgebase in 2021. Nucleic Acids Res 49 (D1): D480-D489, 2021.
13. Trott O and Olson AJ: AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31: 455-461, 2010.
14. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y and Xia R: TBtools: An integrative toolkit developed for interactive analyses of big biological data. Mol Plant 13: 1194-1202, 2020.
15. Martin A, Ochagavia ME, Rabasa LC, Miranda J, Fernandez-de-Cossio J and Bringas R: BisoGenet: A new tool for gene network building, visualization and analysis. BMC Bioinformatics 11: 91, 2010.
16. Wang N, Zhang F, Yang L, Zou J, Wang H, Liu K, Liu M, Zhang H, Xiao X and Wang K: Resveratrol protects against L-arginine-induced acute necrotizing pancreatitis in mice by enhancing SIRT1-mediated deacetylation of p53 and heat shock factor 1. Int J Mol Med 40: 427-437, 2017.
17. Arocho A, Chen B, Ladanyi M and Pan Q: Validation of the 2-DeltaDeltaCt calculation as an alternate method of data analysis for quantitative PCR of BCR-ABL P210 transcripts. Diagn Mol Pathol 15: 56-61, 2006.
18. Peng S, Lu XF, Qi YD, Li J, Xu J, Yuan TY, Wu XY, Ding Y, Li WH, Zhou GQ, *et al*: LCZ696 ameliorates oxidative stress and pressure overload-induced pathological cardiac remodeling by regulating the Sirt3/MnSOD pathway. Oxid Med Cell Longev 2020: 9815039, 2020.
19. Wolf CM: Hypertrophic cardiomyopathy: Genetics and clinical perspectives. Cardiovasc Diagn Ther 9 (Suppl 2): S388-S415, 2019.
20. Halliday BP, Cleland JGF, Goldberger JJ and Prasad SK: Personalizing Risk Stratification for sudden death in dilated cardiomyopathy: The past, present, and future. Circulation 136: 215-231, 2017.
21. Qiao Y, Zhu B, Tian A and Li Z: PEG-coated gold nanoparticles attenuate β-adrenergic receptor-mediated cardiac hypertrophy. Int J Nanomedicine 12: 4709-4719, 2017.
22. Galiniak S, Aebisher D and Bartusik-Aebisher D: Health benefits of resveratrol administration. Acta Biochim Pol 66: 13-21, 2019.
23. Du L, Chen E, Wu T, Ruan Y and Wu S: Resveratrol attenuates hydrogen peroxide-induced aging through upregulation of autophagy in human umbilical vein endothelial cells. Drug Des Devel Ther 13: 747-755, 2019.
24. Zhang X, Lu H, Xie S, Wu C, Guo Y, Xiao Y, Zheng S, Zhu H, Zhang Y and Bai Y: Resveratrol suppresses the myofibroblastic phenotype and fibrosis formation in kidneys via proliferation-related signalling pathways. Br J Pharmacol 176: 4745-4759, 2019.
25. Li W, Yuan G, Pan Y, Wang C and Chen H: Network pharmacology studies on the bioactive compounds and action mechanisms of natural products for the treatment of diabetes mellitus: A review. Front Pharmacol 8: 74, 2017.
26. Zhang W, Bai Y, Wang Y and Xiao W: Polypharmacology in drug discovery: A review from systems pharmacology perspective. Curr Pharm Des 22: 3171-3181, 2016.
27. Liu TH, Tu WQ, Tao WC, Liang QE, Xiao Y and Chen LG: Verification of resveratrol inhibits intestinal aging by down-regulating ATF4/Chop/Bcl-2/Bax signaling pathway: Based on network pharmacology and animal Front Pharmacol 11: 1064, 2020.
28. Liu B, Palmfeldt J, Lin L, Colaço A, Clemmensen KKB, Huang J, Xu F, Liu X, Maeda K, Luo Y and Jäättelä M: STAT3 associates with vacuolar H^+-ATPase and regulates cytosolic and lysosomal pH. Cell Res 28: 996-1012, 2018.
29. Wang W, Wang S, Liu T, Ma Y, Huang S, Lei L, Wen A and Ding Y: Resveratrol: Multi-targets mechanism on neurodegenerative diseases based on network pharmacology. Front Pharmacol 11: 694, 2020.
30. You L, Wang Z, Li H, Shou J, Jing Z, Xie J, Sui X, Pan H and Han W: The role of STAT3 in autophagy. Autophagy 11: 729-739, 2015.
31. Cao X, Li B, Han X, Zhang X, Dang M, Wang H, Du F, Zeng X and Guo C: Soluble receptor for advanced glycation end-products promotes angiogenesis through activation of STAT3 in myocardial ischemia/reperfusion injury. Apoptosis 25: 341-353, 2020.
32. Lu X, Zhu Z, Jiang L, Sun X, Jia Z, Qian S, Li J and Ma L: Matrine increases NKG2D ligand ULBP2 in K562 cells via inhibiting JAK/STAT3 pathway: A potential mechanism underlying the immunotherapy of matrine in leukemia. Am J Transl Res 7: 1838-1849, 2015.
33. Zhang YG, Zhu X, Lu R, Messer JS, Xia Y, Chang EB and Sun J: Intestinal epithelial HMGB1 inhibits bacterial infection via STAT3 regulation of autophagy. Autophagy 15: 1935-1953, 2019.
34. Wang N, Xin H, Xu P, Yu Z and Shou D: Erxian decoction attenuates TNF-α induced osteoblast apoptosis by modulating the Akt/Nrf2/HO-1 signaling pathway. Front Pharmacol 10: 988, 2019.
35. Goncalves GK, Scalzo S, Alves AP, Agero U, Guatimosim S and Reis AM: Neonatal cardiomyocyte hypertrophy induced by endothelin-1 is blocked by estradiol acting on GPER. Am J Physiol Cell Physiol 314: C310-C322, 2018.
36. Wang XQ, Xu ZT, Zhang GP, Hou N, Mo QX, Wei J, Jiang X, Liu Y and Luo JD: Endophilin A2 attenuates cardiac hypertrophy induced by isoproterenol through the activation of autophagy. Am J Transl Res 11: 5065-5075, 2019.
37. Shirakabe A, Zhai P, Ikeda Y, Saito T, Maejima Y, Hsu CP, Nomura M, Egashira K, Levine B and Sadoshima J: Drp1-dependent mitochondrial autophagy plays a protective role against pressure overload-induced mitochondrial dysfunction and heart failure. Circulation 133: 1249-1263, 2016.
38. Zhang Y, Ding Y, Li M, Yuan J, Yu Y, Bi X, Hong H, Ye J and Liu P: MicroRNA-34c-5p provokes isoprenaline-induced cardiac hypertrophy by modulating autophagy via targeting ATG4B. Acta Pharm Sin B 12: 2374-2390, 2022.
39. Luan Y, Luan Y, Feng Q, Chen X, Ren KD and Yang Y: Emerging role of mitophagy in the heart: Therapeutic potentials to modulate mitophagy in cardiac diseases. Oxid Med Cell Longev 2021: 3259963, 2021.
40. Li Y, Chen X, Li P, Xiao Q, Hou D and Kong X: CD47 antibody suppresses isoproterenol-induced cardiac hypertrophy through activation of autophagy. Am J Transl Res 12: 5908-5923, 2020.
41. Wang SY, Ni X, Hu KQ, Meng FL, Li M, Ma XL, Meng TT, Wu HH, Ge D, Zhao J, *et al*: Cilostazol alleviate nicotine induced cardiomyocytes hypertrophy through modulation of autophagy by CTSB/ROS/p38MAPK/JNK feedback loop. Int J Biol Sci 16: 2001-2013, 2020.
42. Xie C, Liu S, Wu B, Zhao Y, Chen B, Guo J, Qiu S and Cao YM: miR-19 promotes cell proliferation, invasion, migration, and EMT by inhibiting SPRED2-mediated autophagy in osteosarcoma cells. Cell Transplant 29: 963689720962460, 2020.
43. Tang Z, Takahashi Y, Chen C, Liu Y, He H, Tsotakos N, Serfass JM, Gebru MT, Chen H, Young MM and Wang HG: Atg2A/B deficiency switches cytoprotective autophagy to non-canonical caspase-8 activation and apoptosis. Cell Death Differ 24: 2127-2138, 2017.
44. Feng Q, Wang H, Pang J, Ji L, Han J, Wang Y, Qi X, Liu Z and Lu L: Prevention of wogonin on colorectal cancer tumorigenesis by regulating p53 nuclear translocation. Front Pharmacol 9: 1356, 2018.
45. Mariño G, Niso-Santano M, Baehrecke EH and Kroemer G: Self-consumption: The interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 15: 81-94, 2014.
46. Valcourt DM, Dang MN, Scully MA and Day ES: Nanoparticle-mediated co-delivery of notch-1 antibodies and ABT-737 as a potent treatment strategy for triple-negative breast cancer. ACS Nano 14: 3378-3388, 2020.
47. Cusack CL, Swahari V, Hampton Henley W, Michael Ramsey J and Deshmukh M: Distinct pathways mediate axon degeneration during apoptosis and axon-specific pruning. Nat Commun 4: 1876, 2013.
48. Acuner Ozbabacan SE, Keskin O, Nussinov R and Gursoy A: Enriching the human apoptosis pathway by predicting the structures of protein-protein complexes. J Struct Biol 179: 338-346, 2012.