Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Apigenin Attenuates Transverse Aortic Constriction-Induced Myocardial Hypertrophy: The Key Role of miR-185-5p/SREBP2-Mediated Autophagy

Na Yan[1], Xianggui Wang[1], Zufang Xu[1], Linling Zhong[1], Jiangyong Yang[2]

[1]Department of Vasculocardiology, Ganzhou People's Hospital, Ganzhou, People's Republic of China; [2]Department of Cardiology, Ganzhou Hospital of Guangdong Provincial People's Hospital, Ganzhou Municipal Hospital, Ganzhou, People's Republic of China

Correspondence: Jiangyong Yang, Department of Cardiology, Ganzhou Hospital of Guangdong Provincial People's Hospital, Ganzhou Municipal Hospital, No. 49, Dagong Road, Ganzhou, 341000, People's Republic of China, Tel +86-797-8208553, Email yangjy19861208@163.com

Introduction: Apigenin is a natural flavonoid compound with promising potential for the attenuation of myocardial hypertrophy (MH). The compound can also modulate the expression of miR-185-5p that both promote MH and suppress autophagy. The current attempts to explain the anti-MH effect of apigenin by focusing on changes in miR-185-5p-mediated autophagy.

Methods: Hypertrophic symptoms were induced in rats using transverse aortic constriction (TAC) method and in cardiomyocytes using Ang II and then handled with apigenin. Changes in myocardial function and structure and cell viability and surface area were measured. The role of miR-185-5p in the anti-MH function of apigenin was explored by detecting changes in autophagic processes and miR-185-5p/SREBP2 axis.

Results: TAC surgery induced weight increase, structure destruction, and collagen deposition in hearts of model rats. Ang II suppresses cardiomyocyte viability and increased cell surface area. All these impairments were attenuated by apigenin and were associated with the restored level of autophagy. At the molecular level, the expression of miR-185-5p was up-regulated by TAC, while the expression of SREBP2 was down-regulated, which was reserved by apigenin both in vivo and in vitro. The induction of miR-185-5p in cardiomyocytes could counteracted the protective effects of apigenin.

Discussion: Collectively, the findings outlined in the current study highlighted that apigenin showed anti-MH effects. The effects were related to the inhibition of miR-185-5p and activation of SREBP, which contributed to the increased autophagy.

Keywords: apigenin, autophagy, miR-185-5p, myocardial hypertrophy, SREBP2

Introduction

Myocardial hypertrophy (MH) is defined as a compensatory response of heart, which is associated with the progression of diverse cardiovascular diseases and will lead to decreased cardiac function.[1] Additionally, when hypertrophic symptoms due to diabetes and hypertension prolong, it will in turn culminate in chronic heart failure or sudden cardiac death.[2] Currently, the mechanism underlying the initiation of MH is only partially elucidated. Studies demonstrate that the pathogenesis of the disorder is closely related to the dysfunctions in gene transcription, calcium regulation, inflammation, oxidative stress, and autophagy.[3–7] Thus, signaling transductions associated with these processes have been considered to be promising targets for the development of novel strategies against MH.

Autophagy is a biological process delivering cytoplasmic material and intracellular organelles to lysosomes for degradation or recycling,[8] which is important for cell survival under nutrient deprivation stress.[9] Therefore, the dysregulation of autophagy has been conceived as the initiation of multiple disorders, including cardiovascular diseases.[10] The disorders in the autophagic process are particularly detrimental to heart tissues in that cardiomyocytes are terminally-differentially cells whose house-keeping functions substantially depend on the regular autophagy.[11] For instance, basal autophagy facilitates cardiomyocytes to

Received: 11 April 2024
Accepted: 21 August 2024
Published: 27 August 2024

© 2024 Yan et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

maintain ATP levels, while disordered autophagy induces the degradation of pivotal organelles and proteins.[12,13] Thus, to prevent cardiovascular system from disorders such as MH, approaches capable of restoring regular autophagy are in great need.

MicroRNAs (miRs) are a class of non-coding RNAs (~20 nt) and closely related to the progression of multiple human diseases.[14] Regarding disorders in myocardial system, miR-21-5p is significantly up-regulated in myocardium of the transverse aortic constriction (TAC) model,[15] and miR-92a-3p is deregulated in myocardial tissues in vascular injury models.[16,17] These previous studies indicate that the modulation of specific miRs may serve as a treatment for myocardial disorders including MH.

Currently, increasing attention is being paid to the management of cardiovascular disorders using natural products. Additionally, the function of compounds with cardiac protective has also been related to the modulation of autophagy. For instance, resveratrol, curcumin, and berberine all show the ability to regulate autophagy via different pathways, such as AMPK, SITR1, or mTOR.[18–20] Apigenin (4', 5, 7-trihydroxyflavone) is a natural flavonoid compound widely distributed in a variety of vegetables and fruits, and its amount is particularly high in chamomile (*Matricaria chamomilla* L.) from the Asteraceae family, which is widely used in the clinic for hypertension by traditional healers in many countries.[21,22] In addition to handling hypertension, apigenin has shown promising potential for different biological applications including the attenuation of MH.[23] For instance, apigenin can improve cardiac hypertrophy through modulating NADPH oxidase-dependent ROS generation and cytokines.[24] Furthermore, the study by Zhu et al showed that the compound could ameliorate hypertension-induced cardiac hypertrophy and down-regulate cardiac hypoxia-inducible factor-lα in rats.[25] The studies collectively confirm the promising protective effects of apigenin against MH, which can be exerted via different mechanisms. However, no previous study has explored the role of autophagy in the anti-MH function of apigenin. Regarding effects of apigenin on autophagy, the study by Xie et al showed that apigenin could alleviate intervertebral disc degeneration via restoring autophagy flux.[26] Moreover, Hsu et al indicated that apigenin ameliorated hepatic lipid accumulation by activating the autophagy pathways.[27] In our previous studies, we have identified that miR-185-5p responded to the treatment of apigenin in a TAC rat model. The miR is reported to be dysregulated in different cardiovascular diseases and promotes the progression of myocardial fibrosis by targeting apelin.[28–30] A downstream effector of miR-185-5p, SREBP2, is a factor exerting function via the modulation of autophagic process.[31] These previous studies together indicated a potential role of autophagy in the anti-MH function of apigenin: the compound might attenuate MH by modulating miR-185-5p/SREBP2-mediated autophagy.

To verify the hypothesis, the expression status of miR-185-5p/SREBP2 and downstream effectors was detected under apigenin in human cardiomyocytes. Then, changes in autophagy were determined both in vitro and in vivo under the administration of apigenin, which would provide valuable information for explaining the mechanism underlying the anti-MH effects of the compound.

Materials and Methods

Transverse Aortic Constriction Model and Experimental Design

Eight-week-old male Sprague-Dawley (SD) rats (weight ~200 g) were purchased from Huafukang Bioscience Co. Inc. (China) and housed with free access to food and water. All procedures were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals (202201421A, Ganzhou Municipal Hospital, China). To induce MH, rats were subjected to the ligation of aorta between the innominate and left common carotid arteries, which would cause left ventricular pressure overload and hypertrophy.[32] The effects of apigenin on the impairments associated with MH were assessed by randomly classified 30 rats into five groups (six for each group, and each experiment was conducted as six independent observations): Sham group, rats were underwent sham TAC surgery without ligation of aorta, and gavaged with normal saline (NS) for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; TAC groups, rats were underwent TAC surgery and gavaged with NS for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; Apigenin L group, rats were underwent TAC surgery, and NS was replaced by 50 mg/kg BW apigenin (>98% purity, Sigma-Aldrich, China);[33] Apigenin H group, rats were underwent TAC surgery, and apigenin dose was set to 100 mg/kg BW.[33]

Cardiac Function Measurement

The hemodynamic parameters of rats were first detected to assess the effects of apigenin on MH-induced cardiac dysfunction. The LVESP and LVEDP values were measured using a noninvasive blood pressure system (XBP 1000, Kent Scientific, Torrington, Conn) with awake rats, and the fractional shortening (FS) was measured using Philips SON05500 system (Philips Ultrasound, Bothell, WA). The rats were then sacrificed using overdose pentobarbital sodium, and heart tissues were collected. After measurement of ratios of heart weight to body weight, the production of CK and LDH in hearts was detected using LDH and CK detecting kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

Histological Examination

Histological changes in heart tissues were first detected with H&E staining.[34] For H&E staining, samples were transected and cut into 1.0 cm × 1.0 cm × 0.5 cm-sized blocks, fixed with neutral formalin solution, and dehydrated with ethanol in conventional gradient. After being embedded in paraffin and cut into slices at 5 μm, the sections were stained with hematoxylin (H9627, Sigma, USA) for 5 min, followed by stained in eosin for 40s. The images were captured under a microscope (LW300LFT-LED, Shanghai Metrology Optoelectronic Technology Co. LTD, China) at 200× magnification. The nucleus of tissues appeared blue and the other parts appeared pink.

The accumulation of collages in heart tissues was further determined using Masson Trichrome Staining:[35] briefly, tissues were separated and fixed in 4% formaldehyde. Then, the tissues were sectioned and then stained with Masson trichrome (Sigma-Aldrich) according to the manufacturer's instructions. The images were captured a microscope (LW300LFT-LED, Shanghai Metrology Optoelectronic Technology Co. LTD, China) at 200× magnification, and the collage-positive areas were quantified using Image J software version 1.46. Collagen fiber was stained blue, cell nuclei were stained black, and the cell cytoplasm was stained red.

Autophagy in heart tissues was then determined via transmission electron microscope (TEM) detection of autophagosome:[36] briefly, liver tissue sections were embedded in Epon resin, and representative areas were chosen for the detection using a FEI Tecnai G2 Spirit Bio TWIN transmission electron microscope (FEI Co., Netherlands) at an acceleration voltage of 120 kV.

Immunofluorescent Assay

For immunofluorescent detection, myocardial tissues were fixed with 4% paraformaldehyde for 15 minutes and permeabilized with 0.1% Triton X-100 for 30 minutes. After three washes with PBS (5 minutes each), sections were blocked in 10% goat serum for 15 minutes. Primary rabbit polyclonal antibodies against specific markers (1:200 dilution) were added, and sections were incubated overnight at 4°C. Staining was performed using a Cy3-labeled secondary antibody (1:200 dilution) for 1 hour in the dark. Following incubation with the secondary antibody, sections were washed and stained with 4.6-diamino-2-phenylindole (DAPI) for 5 minutes at room temperature. After three washes with PBS buffer (5 minutes each), slides were fixed and imaged using fluorescence microscopy (BX53, Olympus) at 200× magnification.

Reverse Transcription Quantitative PCR (RT-qPCR)

To determine the expression level of target molecules, total RNA was extracted for different samples using the TRIzol solution (#15,596,026, Invitrogen). cDNA templates were achieved using a high-capacity cDNA reverse transcription kit (RR047, TaKaRa) and 1 μg RNA was used as the template for qPCR amplification with 2×Power Taq PCR MasterMix (PR1702, Bioteke, China) using ExicyclerTM 96 (BIONEER, South Korea). The primer information was shown in Table S1 and the relative expression level was calculated according to the formula of $2^{-\Delta\Delta ct}$.

Dual Luciferase Assay

The direct interaction between miR-185-5p and 3'UTR of SREBP2 gene was assessed using dual-luciferase assay. Wild-type (WT) SREBP2 and mutant (MUT) SREBP2 plasmid were co-transfected with negative control (NC) mimics or miR-185-5p mimics into cells using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) with renilla

luciferase plasmid as the internal control. Forty-eight hours after the transfection, the luciferase activity was assessed using a Microplate Reader (ELX-800, BIOTEK, USA).

Cell Culture

Rat cardiomyocytes (ZQ0102, Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd, Shanghai, China) were cultured in glucose-free Dulbecco's Modified Eagle Medium (DMEM) in an atmosphere of 95% N_2 and 5% CO_2 at 37°C for 4 h. To induce hypertrophic feature, cells were incubated with Ang II (1 μM) (15,663-7-1, Melonepharma, China) for 48 h.[35] Cells from three to five passages were used for subsequently assays and grouped into four groups (each experiment was conducted as six independent observations): Control group, cardiomyocytes cells; Ang II group, cells incubated with Ang II; Treatment group, cells were pre-treated with apigenin 10 μM for 24 h and then incubated with Ang II; Treatment + Mimic group, cells transfected with miR-185-5p inhibitor (Sango, Shanghai, China) and then subjected to the treatment of apigenin and Ang II.

Cell Viability and Surface Area Measurement

Cell viability was determined using CCK-8 assay: the supernatant of cultures was discarded, and cells were incubated with 10 μL CCK-8 (HY-K0301, MCE, USA) at 37°C for 1 h. The OD value at 450 nm was employed as the representative of cell viability using a Microplate Reader (ELX-800, BIOTEK, USA). Cell average surface area was also determined to evaluate hypertrophic feature under a microscope (BX53, OLUMPUS, Japan) at 400× magnification.

Statistical Analysis

All the data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by post-hoc multiple comparisons using the Tukey method was performed using GraphPad Prism version 6.0 (GraphPad Software, Inc., San Diego, CA) with a significant level of 0.05 (two-tailed p value).

Results

Effects of Apigenin on Cardiac Function in TAC Mice

The effects of apigenin on MH was then evaluated by detecting changes in cardiac function via hemodynamic parameters and cardiac injury indicators. As shown in Figure 1 and Table S2, values of LVESP and FS were substantially reduced by TAC surgery (Figure 1A and B). The differences between Sham and TAC groups were statistically significant ($p < 0.05$). To the contrary, the value of LVEDP was increased by the model induction (Figure 1C; Table S2). The administrations of apigenin of both doses significantly restored the values of these parameters ($p < 0.05$) (Figure 1; Table S2), and the effects were exerted in a dose-dependent manner. Regarding the production of LDH and CK, the levels of the two indicators were first induced by TAC surgery and then suppressed by apigenin in a dose-dependent manner (Figure 1D and E; Table S2). The detection regarding the cardiac function preliminarily verified the impairments of TAC surgery on cardiac function and the cardio-protective effects of apigenin.

Effects of Apigenin on Heart/Body Ratio and Heart Tissue Structure in TAC Mice

The surgery of TAC increased heart/body ratio of mice, which was then suppressed by apigenin of both doses (Figure 2A), indicating the hypertrophic feature of mice hearts. To directly assess the effects of apigenin MH, the histological changes in heart tissues of model rats were detected with H&E staining, Masson staining, and TEM detection, respectively. The TAC surgery-induced tissue deterioration and immune infiltration and increased myofibers cross-sectional area as illustrated by H&E staining, which was attenuated by apigenin of both doses (Figure 2B). The effect of apigenin on collagen deposition was similar: as illustrated by Masson, the TAC surgery increased the production and distribution of collagens in heart tissues, which was inhibited by apigenin (Figure 2C). The TAC-induced damages and collagen deposition in heart tissues were associated with the suppressed autophagy (Figure 2D), while the antifibrotic effect of apigenin also contributed to the increased autophagy.

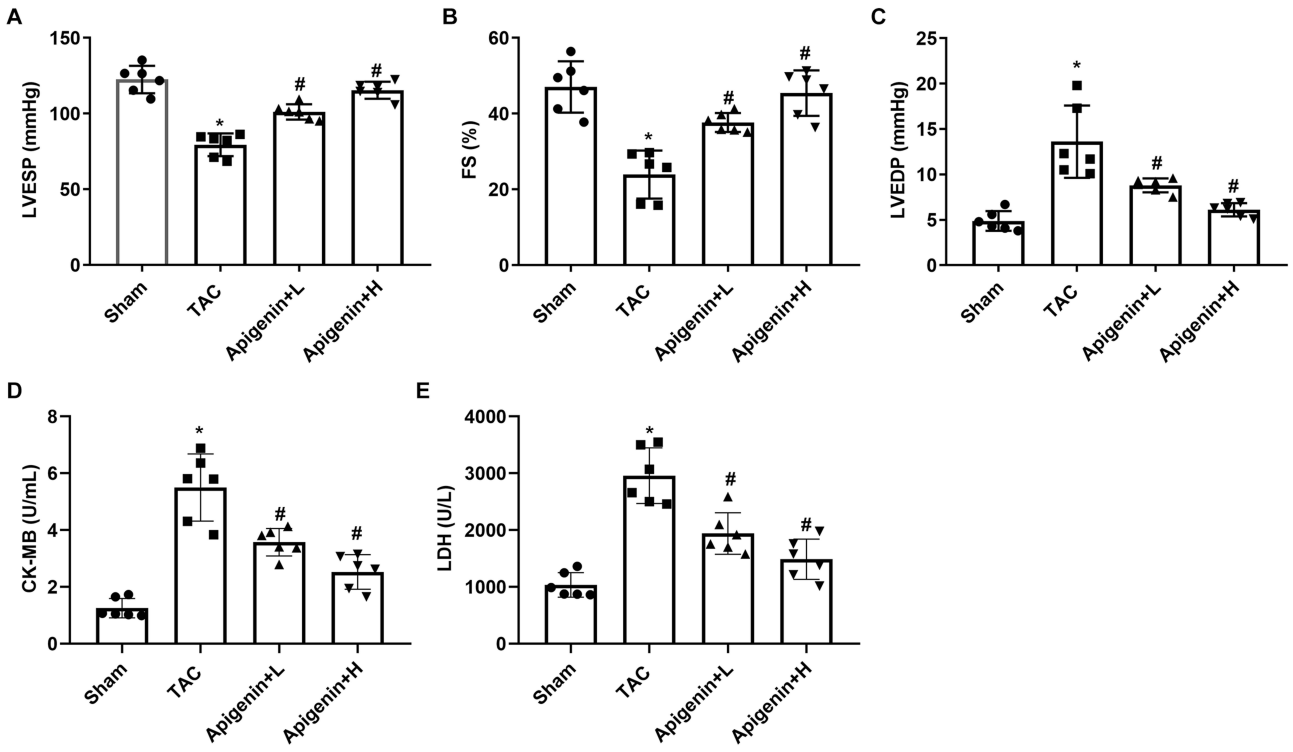

Figure 1 Apigenin attenuates TAC-induced disorders in hemodynamics parameters and heart function in rats (n = 6). (**A**) The quantitative analysis result of LVESP. (**B**) The quantitative analysis result of FS. (**C**) The quantitative analysis result of LVEDP. (**D**) The quantitative analysis result of CK-MB. (**E**) The quantitative analysis result of LDH. Sham group, rats were underwent sham TAC surgery without ligation of aorta, and gavaged with normal saline (NS) for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; TAC groups, rats were underwent TAC surgery, and gavaged with NS for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; Apigenin L group, rats were underwent TAC surgery, and NS was replaced by 50 mg/kg BW apigenin; Apigenin H group, rats were underwent TAC surgery, and apigenin dose was set to 100 mg/kg BW.
Notes: "*" $p < 0.05$ vs Sham group. "#" $P < 0.05$ vs TAC group.

Effects of Apigenin on miR-185-5p/SREBP2 Pathway in TAC Mice

The mechanism underlying the cardio-protective effect of apigenin was explored by detecting changes in miR-185-5p/SREBP2 axis. The establishment of the MH model up-regulated the expression of miR-185-5p level (Figure 3A). The expression patterns of miR-185-5p and SREBP2 were then reversed by apigenin. To confirm the regulation of miR-185-5p on SREBP2, the current study also performed a dual luciferase assay. The data showed that only the co-transfection of miR-185-5p mimics and WT SREBP 3'UTR sequence significantly suppressed the activity of luciferase (Figure 3C), indicating that SREBP2 was a direct downstream effector of miR-185-5p. Correspondingly, the expression level of SREBP2 (Figure 3C) was first suppressed by TAC model and then induced by apigenin of two doses. The effects of apigenin on autophagic process were also then assessed by the changing pattern of LC3-II/LC3-I, Beclin-1, and p62 (Figure 3C). Moreover, the expression and distribution of SREBP2 were further verified by immunofluorescence detection, and the results were similar to that of Western blotting detection (Figure 3D). The regulation of apigenin on miR-185-5p/SREBP2 axis inferred that the cardiac-protective function of apigenin was related to the changes in the signaling transduction.

Effects of Apigenin on Viability, Autophagy and miR-185-5p/SREBP2 Pathway in Ang II-Treated Cardiomyocytes

The cardiac-protective effects of apigenin against hypertrophic symptoms were further verified by inducing the expression of miR-185-5p in vitro (Figure 4A). As shown in Figure 4B and C, the incubation with Ang-II suppressed the viability and increased cell surface area of cardiomyocytes, which was then increased by apigenin ($P < 0.05$). However, the transfection with miR-185-5p mimics counteracted the anti-hypertrophic effects of apigenin (Figure 4B and C), indicating that the

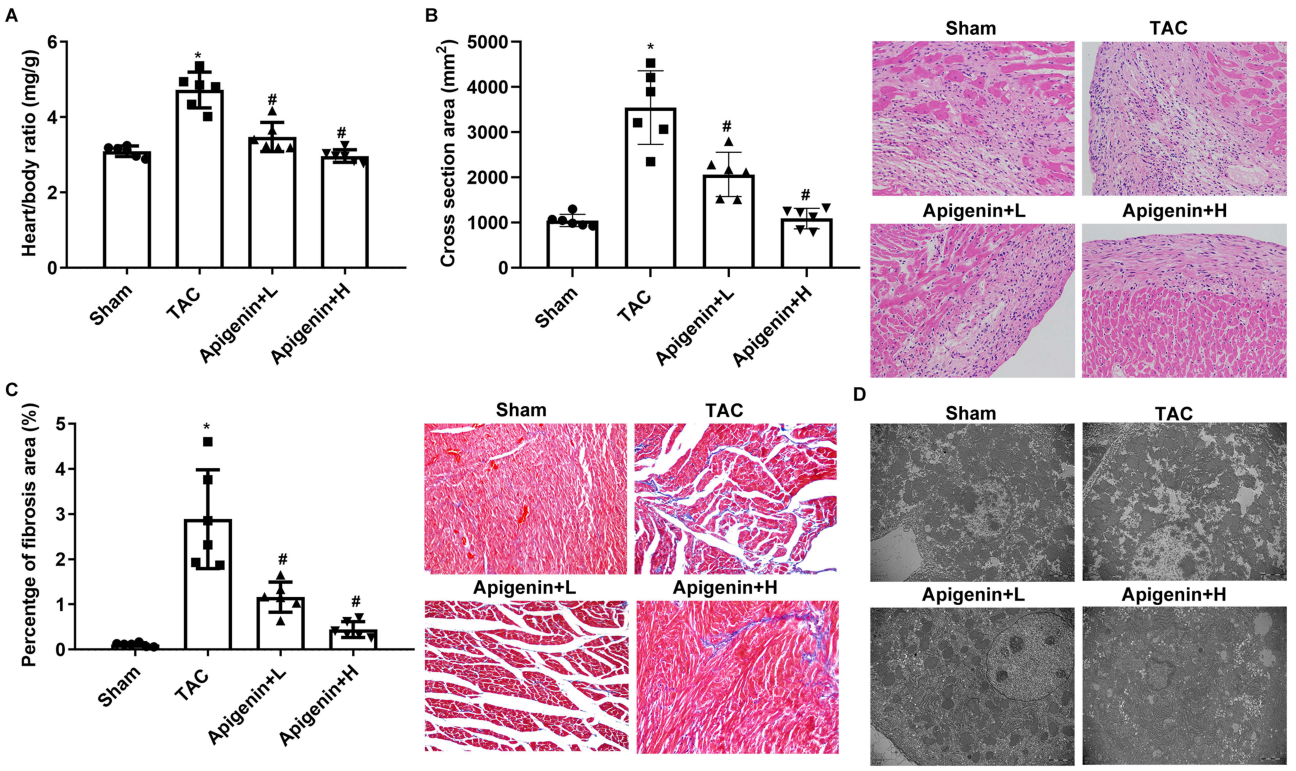

Figure 2 Apigenin suppresses TAC-induced heart weight increase, myocardial hypertrophy, and collagen deposition, and restores autophagy in rat (n = 6). (**A**) The quantitative analysis result of heart/body ratio. (**B**) The quantitative analysis result and representative images of H&E staining. (**C**) The quantitative analysis result and representative images of Masson staining. (**D**) Representative images of TEM detection of autophagy. Sham group, rats were underwent sham TAC surgery without ligation of aorta, and gavaged with normal saline (NS) for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; TAC groups, rats were underwent TAC surgery, and gavaged with NS for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; Apigenin L group, rats were underwent TAC surgery, and NS was replaced by 50 mg/kg BW apigenin; Apigenin H group, rats were underwent TAC surgery, and apigenin dose was set to 100 mg/kg BW.
Notes: "*" $p < 0.05$ vs Sham group. "#" $p < 0.05$ vs TAC group.

suppression of miR-185-5p was indispensable for the protective effect of apigenin on cardiomyocytes. Moreover, the autophagic process was first inhibited by Ang II and then restored by apigenin, which was also re-inhibited by miR-185-5p inhibitor (Figure 4D and E), inferring that the inhibition of miR-185-5p by apigenin contributed to the attenuation of MH via the activation of autophagy.

Discussion

Pathological myocardial hypertrophy is a major risk factor contributing to the progression of heart dysfunction and heart failure. Currently, the mechanism driving the initiation of MH has not been fully understood, many potential theories have been proposed, and disorders in autophagic processes are one intriguing theory for explaining the mechanism driving the progression of MH. For instance, the recent study by Lu et al shows that metrnl ameliorates diabetic cardiomyopathy by deactivating cGAS/STING signaling dependent on LKB1/AMPK/ULK1-mediated autophagy.[37] In the study performed by Yu et al, the authors demonstrate that BMAL1 protects against cardiac hypertrophy by inducing autophagy.[38] Thus, the exploration of the interaction between autophagy and MH may provide valuable information for developing novel anti-MH strategies.

As a process of degradation of excess or damaged organelles or other components in cells with the aid of lysosomes,[39] autophagy can occur in all types of cells and is crucial for maintaining homeostasis of intracellular environment.[40] Therefore, the dysregulation of autophagy is related to the progression of different diseases, such as cancer, neurodegeneration, and diabetes.[41] In the current study, the establishment of TAC model and progression of hypertrophic symptoms were associated with the suppression of autophagy both in rats and in cardiomyocytes, which was in consistence with the previous reports regarding the interaction between autophagy and MH. Additionally, the

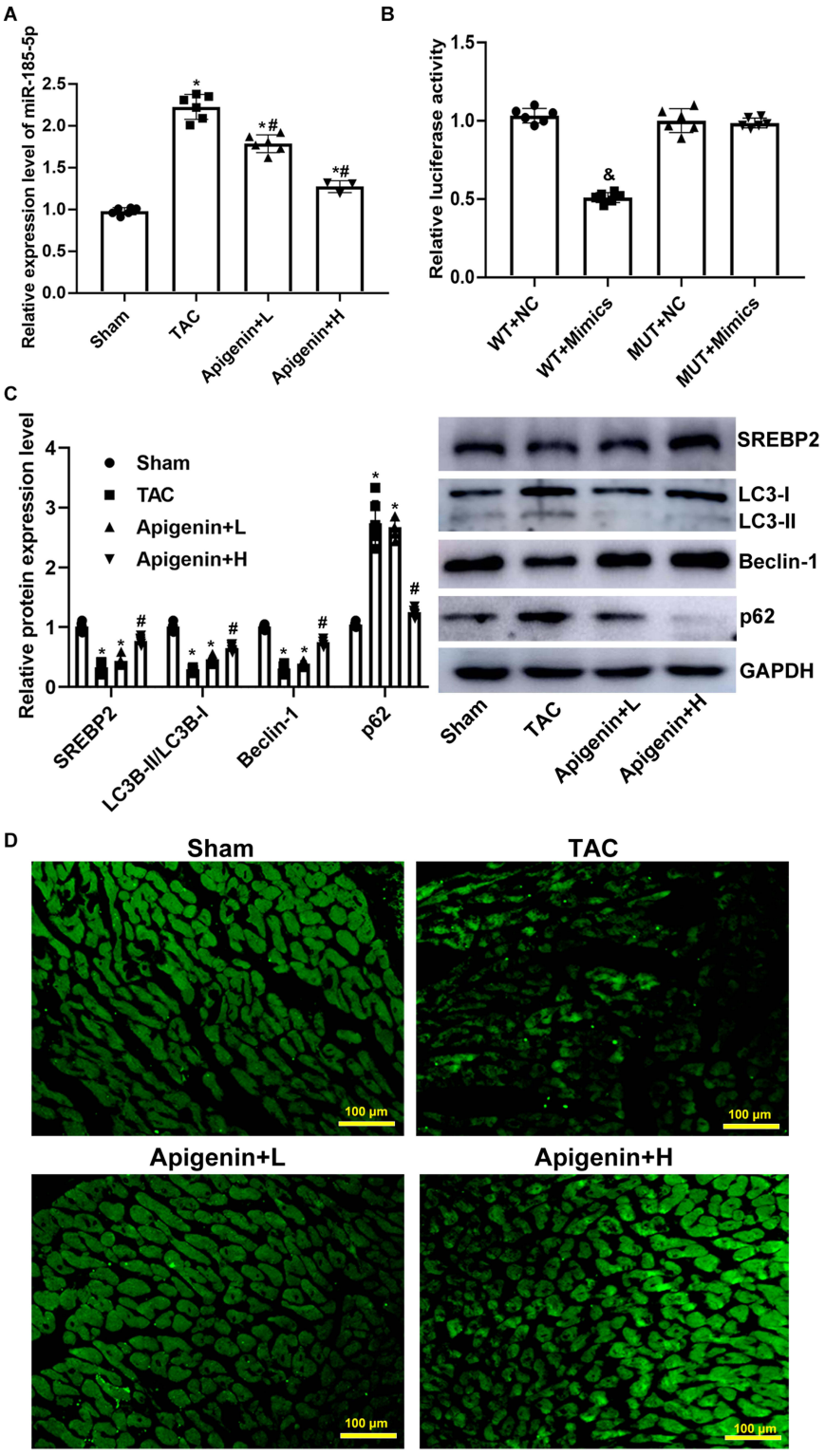

Figure 3 Apigenin suppresses miR-185-5p, while increases SREBP2 expression in heart tissues of TAC rats (n = 6). (**A**) The quantitative analysis result of RT-qPCR detection of miR-185-5p. (**B**) The quantitative analysis result of dual luciferase assay. (**C**) The quantitative analysis result and representative images of Western blotting detection of SREBP2. (**D**) Immunofluorescence detection of SREBP2. Sham group, rats were underwent sham TAC surgery without ligation of aorta, and gavaged with normal saline (NS) for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; TAC groups, rats were underwent TAC surgery, and gavaged with NS for seven days for the surgery and then gavaged with NS for another 14 days after the surgery; Apigenin L group, rats were underwent TAC surgery, and NS was replaced by 50 mg/kg BW apigenin; Apigenin H group, rats were underwent TAC surgery, and apigenin dose was set to 100 mg/kg BW.

Notes: "*" $p < 0.05$ vs Sham group. "#" $p < 0.05$ vs TAC group. "&" $p < 0.05$ vs WT+NC group. Scale bar, 100 μm.

Abbreviations: WT+NC, wild type of 3'UTR of SREBP2 and NC mimics. WT+Mimics, wild type of 3'UTR of SREBP2 and miR-185-5p mimics. MUT+NC, mutant type of 3'UTR of SREBP2 and NC mimics. MUT+Mimics, mutant type of 3'UTR of SREBP2 and miR-185-5p mimics.

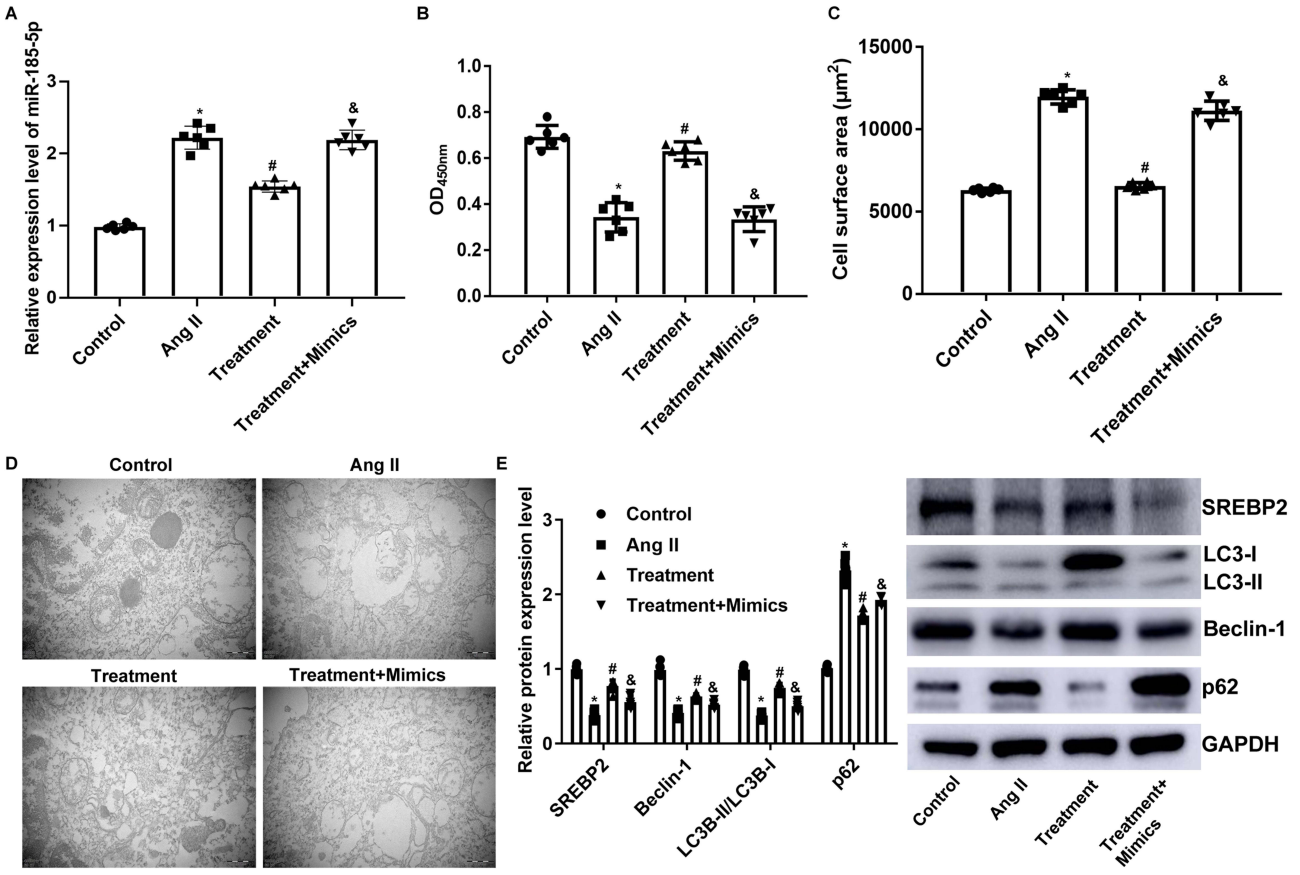

Figure 4 Apigenin increases viability, suppresses cell surface area, restores autophagy in a miR-185-5p-inhibition dependent manner (n = 6). (**A**) The quantitative analysis result of RT-qPCR detection of miR-185-5p. (**B**) The quantitative analysis result of CCK-8 assay. (**C**) The quantitative analysis result of cell surface area. (**D**) Representative images of TEM detection of autophagy. (**E**) The quantitative analysis result and representative images of Western blotting detection of SREBP2 and autophagy indicators. Control group, cardiomyocytes cells; Ang II group, cells incubated with Ang II; Treatment group, cells were pre-treated with apigenin 10 μM for 24 h and then incubated with Ang II; Treatment + Mimics group, cells transfected with miR-185-5p inhibitor and then subjected to the treatment of apigenin and Ang II.
Notes: "*" $p < 0.05$ vs Control group. "#" $p < 0.05$ vs Ang II group. "&" $p < 0.05$ vs Treatment group.

suppression of autophagy was accompanied by the down-regulation of miR-185-5p and up-regulation of SREBP2. Based on the previous reports and our pre-experimental detections, miR-185-5p is dysregulated in different cardiovascular diseases. For example, lncRNA FAF can attenuate hypoxia/ischaemia-induced pyroptosis by sponging miR-185-5p in cardiomyocytes.[29] In the study by Xi et al, the overexpression of miR-185-5p can inhibit BCL-2 protein expression, which consequently increased the ox-LDL-induced human umbilical vein endothelial cells (HUVECs) apoptosis.[28] Additionally, miR-185-5p was a well-characterized pro-fibrosis and pro-hypertrophic factor in myocardium by suppressing the expression of apelin receptor.[30] In the current study, we affirmed the direct interaction between miR-185-5p and SREBP2. The latter protein is a pro-autophagy factor by interacting with PNPLA8.[31] Therefore, the changes of miR-185-5p/SREBP2 axis may be a key signaling transduction mediating the development of MH via the deactivation of autophagy, and the modulation of the axis may serve as a potential strategy for attenuating MH and associated symptoms.

The strategy the current study employed to handle MH and modulate miR-185-5p was apigenin which is a natural flavonoid compound widely distributed. The compound has shown promising potential for the treatments of different disorders including MH.[23,24] Our data showed that the administration of apigenin improved cardiac function, attenuated cardiac hypertrophy induced by TAC surgery, and restored cell viability and surface area in Ang II-treated cardiomyocytes. The treatment process was associated with the up-regulation of SREBP2 and down-regulation of miR-185-5p, which provided a potential explanation for the anti-MH function of apigenin. To further verify the mechanism, the level of miR-185-5p was induced in apigenin-treated cardiomyocytes, and it was found that the inhibition of miR-185-5p was indispensable for the function of apigenin. The changes in miR-185-5p/SREBP2 axis under apigenin treatment in vitro

and in vivo further connected the anti-MH function of the compound to the activation of autophagy, which provided valuable information for the future application of the compounds in the clinical handling of MH.

Collectively, the data in the current study preliminarily reveal the mechanism mediating the anti-MH effect of apigenin: the miR-185-5p level is suppressed by apigenin, and contributes to the activation of SREBP-mediated autophagy, which finally leads to the attenuation of hypertrophic symptoms. However, the interaction between miR-185-5p and apigenin was only implicitly demonstrated, and other miRs might also be involved in the treatment effect of apigenin on MH. Thus, more comprehensive studies are needed in the future to promote the development of anti-hypertrophic therapies: for instance, comprehensively evaluation of the response of different miRs to the treatment of apigenin in MH model, summary of the shared downstream effectors by different miRs, prediction of the interaction between specific downstream effectors and apigenin, which will provide an explicit explanation to the mechanism underlying the anti-MH function of apigenin as well as providing more therapeutic targets for the treatment of MH.

Data Sharing Statement

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

Ethics Statement

All procedures were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals (202201421A, Ganzhou Municipal Hospital, China).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Katz AM, Katz AM. Cardiomyopathy of overload. A major determinant of prognosis in congestive heart failure. *New Engl J Med*. 1990;322(2):100–110. doi:10.1056/nejm199001113220206
2. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *New Engl J Med*. 1990;322(22):1561–1566. doi:10.1056/nejm199005313222203
3. Maillet M, van Berlo JH, Molkentin JD. Molecular basis of physiological heart growth: fundamental concepts and new players. *Nat Rev Mol Cell Biol*. 2013;14(1):38–48. doi:10.1038/nrm3495
4. van Berlo JH, Maillet M, Molkentin JD. Signaling effectors underlying pathologic growth and remodeling of the heart. *J Clin Invest*. 2013;123(1):37–45. doi:10.1172/jci62839
5. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. *Pharmacol Ther*. 2010;128(1):191–227. doi:10.1016/j.pharmthera.2010.04.005
6. Tham YK, Bernardo BC, Ooi JY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. *Arch Toxicol*. 2015;89(9):1401–1438. doi:10.1007/s00204-015-1477-x
7. Shimizu I, Minamino T. Physiological and pathological cardiac hypertrophy. *J Mol Cell Cardiol*. 2016;97:245–262. doi:10.1016/j.yjmcc.2016.06.001
8. Yonekawa T, Thorburn A. Autophagy and cell death. *Essays in Biochemistry*. 2013;55:105–117. doi:10.1042/bse0550105
9. Nikoletopoulou V, Markaki M, Palikaras K, Tavernarakis N. Crosstalk between apoptosis, necrosis and autophagy. *BBA*. 2013;1833(12):3448–3459. doi:10.1016/j.bbamcr.2013.06.001
10. Galluzzi L, Pietrocola F, Levine B, Kroemer G. Metabolic control of autophagy. *Cell*. 2014;159(6):1263–1276. doi:10.1016/j.cell.2014.11.006

11. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science*. 2009;324(5923):98–102. doi:10.1126/science.1164680
12. Ma S, Wang Y, Chen Y, Cao F. The role of the autophagy in myocardial ischemia/reperfusion injury. *BBA*. 2015;1852(2):271–276. doi:10.1016/j.bbadis.2014.05.010
13. Li ZL, Lerman LO. Impaired myocardial autophagy linked to energy metabolism disorders. *Autophagy*. 2012;8(6):992–994. doi:10.4161/auto.20285
14. Bang C, Batkai S, Dangwal S, et al. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *J Clin Invest*. 2014;124(5):2136–2146. doi:10.1172/jci70577
15. Ramanujam D, Schön AP, Beck C, et al. MicroRNA-21-dependent macrophage-to-fibroblast signaling determines the cardiac response to pressure overload. *Circulation*. 2021;143(15):1513–1525. doi:10.1161/circulationaha.120.050682
16. Hinkel R, Penzkofer D, Zühlke S, et al. Inhibition of microRNA-92a protects against ischemia/reperfusion injury in a large-animal model. *Circulation*. 2013;128(10):1066–1075. doi:10.1161/circulationaha.113.001904
17. Bonauer A, Carmona G, Iwasaki M, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science*. 2009;324(5935):1710–1713. doi:10.1126/science.1174381
18. Park D, Jeong H, Lee MN, et al. Resveratrol induces autophagy by directly inhibiting mTOR through ATP competition. *Sci Rep*. 2016;6(1):21772. doi:10.1038/srep21772
19. Kim JY, Cho TJ, Woo BH, et al. Curcumin-induced autophagy contributes to the decreased survival of oral cancer cells. *Arch Oral Biol*. 2012;57(8):1018–1025. doi:10.1016/j.archoralbio.2012.04.005
20. Wang J, Qi Q, Feng Z, et al. Berberine induces autophagy in glioblastoma by targeting the AMPK/mTOR/ULK1-pathway. *Oncotarget*. 2016;7(41):66944–66958. doi:10.18632/oncotarget.11396
21. Olorunnisola O, Bradley G, Afolayan A. Ethnobotanical information on plants used for the management of cardiovascular diseases in Nkonkobe Municipality, South Africa. *J MED PLANTS RES*. 2011;5(17):4256–4260.
22. Neamsuvan O, Komonhiran P, Boonming K. Medicinal plants used for hypertension treatment by folk healers in Songkhla province, Thailand. *J Ethnopharmacol*. 2018;214:58–70. doi:10.1016/j.jep.2017.11.032
23. Hostetler GL, Ralston RA, Schwartz SJ. Flavones: food sources, bioavailability, metabolism, and bioactivity. *Adv Nutri*. 2017;8(3):423–435. doi:10.3945/an.116.012948
24. Gao HL, Yu XJ, Hu HB, et al. Apigenin improves hypertension and cardiac hypertrophy through modulating NADPH oxidase-dependent ROS generation and cytokines in hypothalamic paraventricular nucleus. *Cardiovasc Toxicol*. 2021;21(9):721–736. doi:10.1007/s12012-021-09662-1
25. Zhu ZY, Gao T, Huang Y, Xue J, Xie ML. Apigenin ameliorates hypertension-induced cardiac hypertrophy and down-regulates cardiac hypoxia inducible factor-lα in rats. *Food & Funct*. 2016;7(4):1992–1998. doi:10.1039/c5fo01464f
26. Xie C, Shi Y, Chen Z, et al. Apigenin alleviates intervertebral disc degeneration via restoring autophagy flux in nucleus pulposus cells. *Front Cell Develop Biol*. 2021;9:787278. doi:10.3389/fcell.2021.787278
27. Hsu MC, Guo BC, Chen CH, Hu PA, Lee TS. Apigenin ameliorates hepatic lipid accumulation by activating the autophagy-mitochondria pathway. *J Food & Drug Anal*. 2021;29(2):240–254. doi:10.38212/2224-6614.3269
28. Xi X, Zheng X, Zhang R, Zeng L. Upregulation of circFOXP1 attenuates inflammation and apoptosis induced by ox-LDL in human umbilical vein endothelial cells by regulating the miR-185-5p/BCL-2 axis. *Can J Physiol Pharmacol*. 2022;100(11):1045–1054. doi:10.1139/cjpp-2020-0764
29. Gu J, Shi JZ, Wang YX, et al. LncRNA FAF attenuates hypoxia/ischaemia-induced pyroptosis via the miR-185-5p/PAK2 axis in cardiomyocytes. *J Cell & Mol Med*. 2022;26(10):2895–2907. doi:10.1111/jcmm.17304
30. Lin R, Rahtu-Korpela L, Szabo Z, et al. MiR-185-5p regulates the development of myocardial fibrosis. *J Molecular & Cellular Cardiol*. 2022;165:130–140. doi:10.1016/j.yjmcc.2021.12.011
31. Kim KY, Jang HJ, Yang YR, et al. SREBP-2/PNPLA8 axis improves non-alcoholic fatty liver disease through activation of autophagy. *Sci Rep*. 2016;6(1):35732. doi:10.1038/srep35732
32. Hu P, Zhang D, Swenson L, Chakrabarti G, Abel ED, Litwin SE. Minimally invasive aortic banding in mice: effects of altered cardiomyocyte insulin signaling during pressure overload. *Am J Physiol Heart Circulatory Physiol*. 2003;285(3):H1261–9. doi:10.1152/ajpheart.00108.2003
33. Quan W, Ma S, Zhu Y, Shao Q, Hou J, Li X. Apigenin-7-O-β-d-(6″-p-coumaroyl)-glucopyranoside reduces myocardial ischaemia/reperfusion injury in an experimental model via regulating the inflammation response. *Pharm Biol*. 2020;58(1):80–88. doi:10.1080/13880209.2019.1701043
34. Fan C, Wang Q, Chen Y, Ye T, Fan Y. Puerarin from pueraria lobate attenuates ischemia-induced cardiac injuries and inflammation in vitro and in vivo: the key role of miR-130a-5p/HMGB2 pathway. *Chem Biol & Drug Des*. 2023;101(4):952–961. doi:10.1111/cbdd.14204
35. Zhang X, Qin X. CTRP3/AMPK pathway plays a key role in the anti-hypertrophic effects of cyanidin-3-O-glucoside by inhibiting the inflammatory response. *Adv Clin Exp Med*. 2023. doi:10.17219/acem/172546
36. Yin Q, Yan R, Wang Y, Yu J. Gastrodin from Gastrodia elata attenuates acute myocardial infarction by suppressing autophagy: key role of the miR-30a-5p/ATG5 pathway. *J Funct Foods*. 2023;102:105429. doi:10.1016/j.jff.2023.105429
37. Lu QB, Ding Y, Liu Y, et al. Metrnl ameliorates diabetic cardiomyopathy via inactivation of cGAS/STING signaling dependent on LKB1/AMPK/ULK1-mediated autophagy. *J Adv Res*. 2022;51:161–179. doi:10.1016/j.jare.2022.10.014
38. Yu L, Ren L, Dong L. BMAL1 plays a critical role in the protection against cardiac hypertrophy through autophagy in vitro. *BMC Cardiovasc Disord*. 2022;22(1):381. doi:10.1186/s12872-022-02822-3
39. Bolt AM, Byrd RM, Klimecki WT. Autophagy is the predominant process induced by arsenite in human lymphoblastoid cell lines. *Toxicol Appl Pharmacol*. 2010;244(3):366–373. doi:10.1016/j.taap.2010.01.019
40. Fang L, Zhou Y, Cao H, et al. Autophagy attenuates diabetic glomerular damage through protection of hyperglycemia-induced podocyte injury. *PLoS One*. 2013;8(4):e60546. doi:10.1371/journal.pone.0060546
41. Bai J, Yao X, Jiang L, et al. Taurine protects against As2O3-induced autophagy in livers of rat offsprings through PPARγ pathway. *Sci Rep*. 2016;6(1):27733. doi:10.1038/srep27733

Drug Design, Development and Therapy

Dovepress

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/drug-design-development-and-therapy-journal