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Puerarin prevents cardiac hypertrophy induced by pressure overload through activation of autophagy

Bei Liu [a], Zhiye Wu [a], Yunpeng Li [a], Caiwen Ou [a], Zhenjun Huang [b], Jianwu Zhang [a], Peng Liu [a], Chengfeng Luo [b, **], Minsheng Chen [a, *]

[a] Department of Cardiology, Zhu Jiang Hospital of Southern Medical University, China
[b] Department of Cardiology, The Second Affiliated Hospital of Guangzhou Medical University, China

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ABSTRACT

This study aimed to explore the effects of puerarin on autophagy in cardiac hypertrophy. Decreased 5′-adenosine monophosphate kinase (AMPK) activity alone with inhibited autophagy could be detected in rats within 3 weeks after aortic banding (AB). Puerarin treatment for 3 weeks in AB rats significantly restored autophagy. Administration of puerarin for 6 weeks effectively restricted cardiomyocyte hypertrophy and apoptosis. In an *in vitro* study, similar anti-hypertrophy and anti-apoptosis effects of puerarin on isoprenaline-induced H9c2 cells were also observed. After inhibition of autophagy by pretreatment with 3-methyladenine, the protective effects of puerarin were blocked. Further *in vivo* study demonstrated that puerarin significantly enabled phosphorylation of 5′-AMPK to be activated, subsequently inhibiting expression of the mammalian target of rapamycin (mTOR) target proteins S6 ribosomal protein and 4E-binding protein 1. All these data indicate that puerarin exerts protective effects against cardiomyocyte hypertrophy and apoptosis, partly by restoration of autophagy through AMPK/mTOR-mediated signaling.

1. Introduction

During the early period of cardiac hypertrophy, a shift to raised ventricular tension in response to increased neurohumoral activation and hemodynamic load, which is characterized by increased protein synthesis and hypertrophic sarcomere, is the main compensatory mechanism [1,2]. However, if the stressors are sustained, the hypertrophic cardiomyocytes progressively die, ultimately leading to heart failure.

Autophagy is a conserved process that facilitates the degradation of injured long-lived proteins and organelles in lysosomes, and has been shown to be involved in cardiovascular disease [3]. The current view is that activated autophagy is an adaptive process with pro-survival effects in specific contexts, while dysregulation of autophagy can cause pathological disease [4]. Accumulated data have shown that dysfunction of autophagy is associated with development of cardiac hypertrophy [2,5]. Importantly, activation of autophagy by pharmacotherapy or gene modification can retard development of hypertrophy and protect cardiac function [6,7]. Thus, therapy to preserve autophagic regulation may represent a new and useful strategy for management of cardiac hypertrophy. As the major bioactive ingredient derived from the root of *Pueraria lobata* (Kudzu), puerarin, has been approved by the State Food and Drug Administration in China for clinical therapy in cardiovascular and other diseases [8]. Nevertheless, the detailed mechanisms of puerarin in cardiac hypertrophy remain unclear, especially as to whether puerarin exerts a preventive effect on cardiac hypertrophy by regulation of autophagy. Thus, in the present study, we aimed to determine whether puerarin can retard cardiac hypertrophy by stimulating autophagy, and explored the potential underlying mechanisms.

2. Materials and methods

2.1. Materials

See supplementary material.

* Corresponding author. Department of Cardiology, Zhujiang Hospital of Southern Medical University, No. 1023 Industrial Road, Guangzhou, 510280, China.
** Corresponding author. Department of Cardiology, The Second Affiliated Hospital of Guangzhou Medical University, No. 250 Chang Gang Dong Road, Guangzhou, 510260, China.
E-mail addresses: rocenphone@hotmail.com (C. Luo), minshengchengz@163.com (M. Chen).

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2.2. Animal model

The animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* (US and National Institutes of Health). Male Sprague–Dawley rats weighting 80–100 g (Central Animal Care Services at Southern Medical University) were used. Cardiac hypertrophy was induced by descending AB [2]. Rats assigned to the sham-operated (SO) group underwent a similar procedure except for the arterial ligation. Subcutaneous injection of puerarin (100 mg/kg/day) or rapamycin (1.2 mg/kg/day) was started once the arterial banding was confirmed by echocardiography immediately after the AB procedure. Rats in the SO group received an equal volume of normal saline.

2.3. Echocardiography

Following anesthetization with chloral hydrate, transthoracic two-dimensionally guided M-mode echocardiography was performed every week after the AB procedure by an experienced technician blinded to the study groups, using an IE33 echocardiographic system (Philips Medical Systems, Nederland BV).

2.4. Organ weight and histological analysis

Heart weight to body weight ratio (HW/BW) and left ventricular mass to body weight ratio (LWm/BW) were calculated. Myocyte cross-sectional area was used for evaluation of the degree of left ventricular (LV) hypertrophy. Sections were stained with H&E and examined under a light microscope (AMG EVOS FL), and the myocyte area was measured with Image Pro Plus (v6.0; Media Cybernetics, Carlsbad, CA, USA). Fibrosis was assessed by staining sections with a Masson trichrome kit according to the manufacturer's instructions.

2.5. Immunofluorescence microscopy

After the paraffin wax sectioning was finished, antigen retrieval was performed using EDTA buffer solution (pH 8.0). The dried sections were washed with PBS, blocked with 3% bovine serum albumin (BSA) for 30 min at room temperature, and then incubated with the primary antibodies, followed by the fluorescent secondary antibodies. The sections were imaged with an inverted fluorescence microscope (AMG EVOS FL). Green fluorescence was considered a marker of LC3 positivity.

2.6. Cell culture and treatment

H9c2 cardiac myoblast cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Upon reaching 50–60% confluence, the cells were treated with ISO (1 μM) or puerarin (1 μM, 5 μM, 10 μM, 20 μM, or 40 μM, dissolved in dimethyl sulfoxide [DMSO]), alone or in combination. Positive control groups were exposed to rapamycin (100 nM) in normal medium containing ISO (1 μM). For inhibitor experiments, cells were pretreated with the selective autophagy inhibitor 3-MA (10 mM) for 1 h, or transfected with AMPK siRNA (AMPK-si) for 48 h. For observation of autophagic flux, H9c2 cells were pretreated with CQ (10 μM) for 16 h with or without other treatments.

2.7. Western blot analysis

Western blot assay was performed as follows. Briefly, the supernatants of the tissue or cell lysates were collected after centrifugation. Bradford assay was used to measure the protein concentration. Protein samples (20–25 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were probed with primary antibodies overnight. Diluted secondary antibodies were used to detect the corresponding primary antibodies. Further analysis was carried out using Image Pro Plus v6.0 to quantify the protein bands.

2.8. Quantitative real-time reverse transcription–polymerase chain reaction

Trizol reagent was used to extract RNA from LV tissue or H9c2 cells. cDNA was synthesized for real-time polymerase chain reaction (PCR) analysis. The PCR amplifications were quantified and the results were normalized against 18S gene expression. The PCR primer sequences used in this study were as follows: 18S, 5′-ACCGCAGCTAGGAATAATGGA-3′ (forward), 5′-GCCTCAGTTCCGAAAACCA-3′ (reverse); ANP, 5′-GGGGGTAGGATTGACAGGAT-3′ (forward), 5′-CTCCAGGAGGGTATTCACCA-3′ (reverse); β-MHC, 5′-CCTCGCAATATCAAGGGAAA-3′ (forward), 5′-TACAGGTGCATCAGCTCCAG-3′ (reverse). The $^{2\Delta\Delta}$CT method was used to calculate the relative expression levels of mRNA.

2.9. Apoptosis assays

Apoptosis in heart sections was measured by TUNEL staining. An Annexin V-FITC apoptosis detection kit was used to detect apoptosis in cultured H9c2 cells. Hoechst 33342 staining was also carried out to detect cell apoptosis. Cells with fragmented or condensed nuclei were considered apoptotic cells.

2.10. Measurements of cell size

After incubation with DAPI for 15 min, H9c2 cells were incubated with Alexa Fluor 555 phalloidin (1:35 dilution) for 30 min at 37 °C. A fluorescence microscope was used to visualize the cardiomyocytes and take pictures. Image Pro Plus software was used to calculate the cell surface area, with 100 cells from selected fields measured randomly in each group.

2.11. Visualization of autophagic vacuoles

H9c2 cells were transfected with GFP-LC3 plasmid following the manufacturer's instructions [9]. After the treatments, the GFP-LC3 punctate dot structures in individual live H9c2 cells were imaged using a fluorescence microscope. Autophagy was quantified by calculating the percentage of GFP-LC3-positive autophagic vacuoles in cells with LC3 punctate dots.

2.12. Statistical analyses

Continuous data were expressed as the means ± standard error (SEM). The differences in means between the groups were evaluated using one-way analysis of variance (ANOVA) test, followed by a *post hoc* Tukey test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. LC3B expression and AMPK inhibiting in hypertrophic myocardium in vivo

Reduced LC3B-II and increased p62 protein were observed in AB rats compared with SO rats at week 2 and 3 post-operative, respectively, while at week 6, the expression of LC3-II was much more increased in AB rats than in SO rats. By contrast, p62 was

significantly less in AB rats than in SO rats. Beclin1 was similar in all groups before week 3 post-AB procedure, but it was significantly higher in AB rats at week 6 post-procedure compared with SO rats. The Thr172 phosphorylation of AMPK showed similar changes to those of LC3B-II in AB rats over time (Fig. 1A and B).

3.2. Puerarin restored autophagy though activation of AMPK in vivo

As shown in Fig. 1C and D, compared with rats in the SO group, those in the AB group showed decreased LC3B-II, accompanied by increased p62. Decreased phosphorylation of AMPK at Thr172 was also observed at week 3 post-procedure. In AB rats, 3 weeks of puerarin therapy significantly restored LC3B-II, and this was accompanied by decreased p62. Moreover, AMPK that was reduced in the AB group was also restored in the puerarin therapy group. Either AB alone or AB combined with puerarin therapy had limited influence on Beclin1 protein expression at week 3 post-procedure. The representative images of LC3-positive fluorescence (green) also indicated that the AB procedure reduced LC3 at week 3, and that puerarin therapy resulted in the restoration of LC3 (Fig. 1E). Rapamycin, a classic activator of autophagy, was chosen to treat AB rats as a positive control group; this group had similar results in terms of changed autophagic markers and AMPK activity as those in the puerarin-treated group.

3.3. Puerarin prevented cardiac hypertrophy and remodeling

As shown in Fig. 2A and B, impaired LV function indicated by reduced left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) was not observed in AB rats at week 6. However, hypertrophic indicators such as left ventricular posterior wall end-diastolic thickness (LVPWd), left ventricular posterior wall end-systolic thickness (LVPWs), interventricular septum end-diastolic thickness (IVSd), and interventricular septum end-systolic thickness (IVSs) increased significantly in the AB group compared with the SO group. After 6 weeks of therapy with either puerarin or rapamycin, those indicators decreased significantly compared with untreated AB rats (Fig. 2C). H&E staining analysis of cardiac cross-sections revealed that the increased myocyte area noted in AB rats was reduced by either puerarin or rapamycin treatment (Fig. 2D and E). Analysis with Masson's stain demonstrated that both interstitial and perivascular fibrosis were increased in rats with AB, and both puerarin and rapamycin treatment significantly reduced myocyte area and fibrosis compared with AB rats (Fig. 2F and G). Similar changes in HW/BW and LVm/BW were also observed in these groups (Fig. 2H). Besides, the increases in ANP and β-MHC mRNA induced by AB surgery were also significantly prevented in either puerarin-treated or rapamycin-treated rats (Fig. 2I).

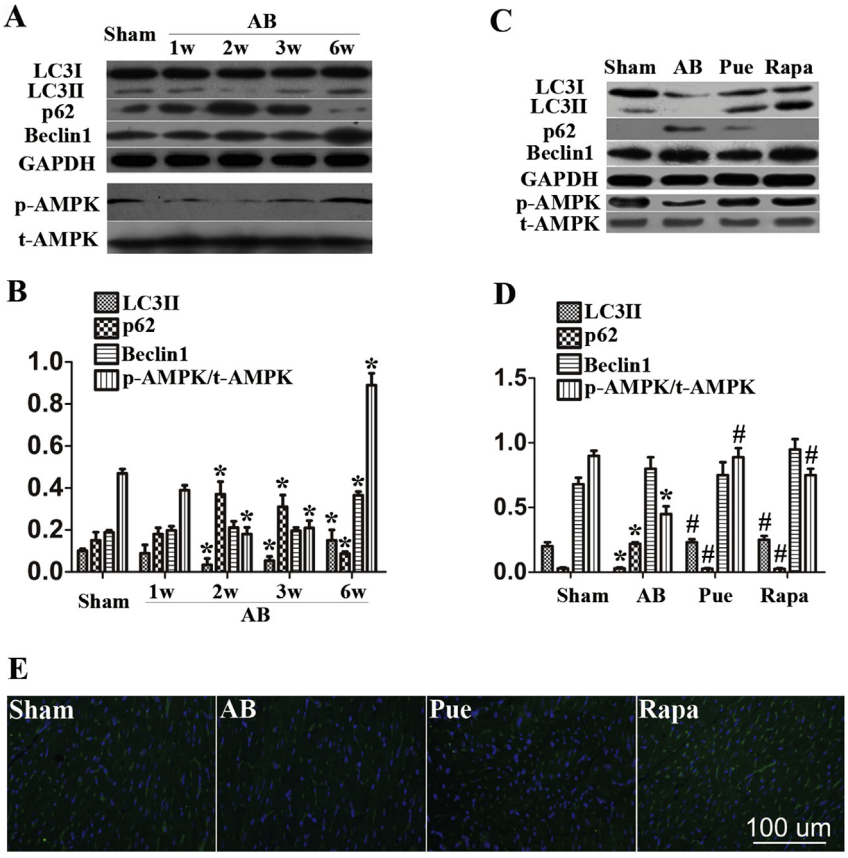

Fig. 1. Effects of puerarin on autophagy *in vivo* study. *A* and *B*: Representative immunoblot and quantitative analysis of LC3B, p62, Beclin1, GAPDH, p-AMPK and total AMPK levels in hearts subjected to AB or sham operation for the indicated time. *C* and *D*: Representative immunoblot and quantitative analysis of LC3B, p62, Beclin1, GAPDH, p-AMPK, and total AMPK levels in rats in the sham, AB, puerarin, and rapamycin groups, respectively. *E*: Representative images of LC3-positive fluorescence (green). Blue fluorescence indicates DAPI-stained nuclei. AB, aortic banding group; Pue, puerarin-treated aortic banding group; Rapa, rapamycin-treated aortic banding (positive control) group. $^{*}P < 0.05$ vs Sham group; $\#P < 0.05$ vs AB group. Each of the experiments was repeated four times, n = 4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

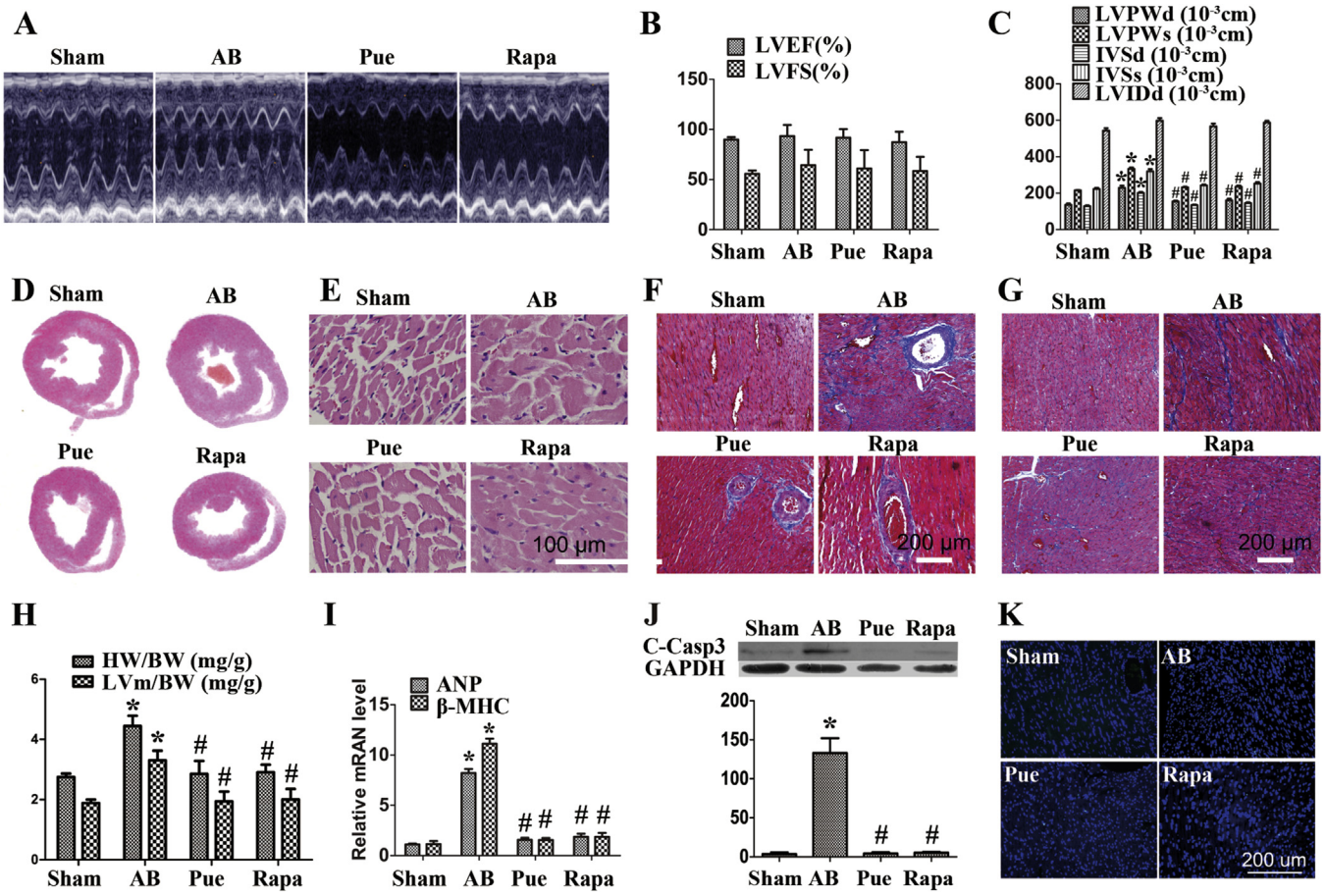

Fig. 2. Evidence of attenuation of AB-induced LV hypertrophy and apoptosis at week 6. Recordings were shown from 6 rats in each group. *A*: Representative pictures of echocardiography; *B*: left ventricular function; *C*: indexes of cardiac structure. *D* and *E*: H & E staining; *F* and *G*: representative photomicrographs of Masson trichrome staining for interstitial fibrosis and perivascular fibrosis respectively; *H*: HW/BW and LVm/BW; *I*: quantitative reverse transcription PCR analysis of the hypertrophy-related genes *ANP* and *β-MHC*; *J*: Representative immunoblot and quantitative analysis of cleaved caspase 3 from each group; *K*: representative images of TUNEL staining. Apoptotic cells (green) in heart sections from each group were labeled by TUNEL staining. AB, aortic banding group; Pue, puerarin-treated aortic banding group; Rapa, rapamycin-treated aortic banding (positive control) group. $^{*}P < 0.05$ vs. sham group; $^{\#}P < 0.05$ vs. AB group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Puerarin protected against cardiomyocyte apoptosis

A marked increase in cardiomyocyte apoptosis, as indicated by increased cleaved caspase 3 protein, was detected at week 6 in AB rats compared with SO rats (Fig. 2J). TUNEL-positive cells were seldom identified in SO rats, but were markedly increased in AB rats, and this was blocked significantly in both puerarin-treated and rapamycin-treated AB rats (Fig. 2K).

3.5. Autophagy dysfunction was associated with inhibited AMPK in vitro

Treatment with ISO 1 μM for at least 12 h significantly induced an increase in LC3B-II accompanied by activation of AMPK, as indicated by increased phosphorylation of AMPK at Thr127. However, decreased LC3B-II in H9c2 was detected within 6 h post-ISO treatment, and the changes in AMPK were exactly the same (Fig. 3A).

3.6. Puerarin restored autophagy activity inhibited by ISO in vitro

Similar to rapamycin, puerarin pretreatment for 24 h followed by co-culture with ISO for 6 h could dose-dependently increase LC3B-II compared with ISO treatment alone (Fig. 3B). Reduced LC3-II protein levels under basal conditions could be detected in the cells transfected with AMPK-Si. Importantly, Puerarin treatment restored LC3-II level in the cells transfected with control siRNA but failed to do so in the cells transfected with AMPK-Si (Fig. 3C). Pretreatment with CQ for 16 h resulted in increased LC3B-II accumulation in H9c2 cells without any other treatment, and this was attenuated by ISO treatment. Cells pretreated with both puerarin (20 μM) and CQ, followed by co-culture with ISO, showed an additional increase in the number of GFP-LC3 puncta (Fig. 3D and E).

3.7. Puerarin prevented H9c2 hypertrophy through promoting autophagy in vitro

Compared with the H9c2 cells in the control group, administration of ISO for 48 h markedly increased the cell area (Fig. 3F and G) and the expression of ANP and β-MHC mRNA (Fig. 3H), which was significantly restrained by either puerarin or rapamycin therapy. After suppression of autophagy by pre-treating cells with 3-MA, puerarin failed to reduce the cell surface area and the mRNA expression levels of ANP and β-MHC in the presence of ISO.

3.8. Puerarin protected H9c2 against apoptosis by promoting autophagy in vitro

ISO administration to H9c2 cells significantly induced cardiomyocyte apoptosis, as determined by higher levels of apoptotic cells in ISO treated cells than normal control cells (Fig. 4A). Hoechst 33342 fluorescent stain revealed the pro-apoptotic effects of ISO, which was characterized by increased numbers of white hyperchromatic nuclei (Fig. 4B). Also, compared with normal control cells, increased protein level of cleaved caspase 3 in ISO treated cells could be observed (Fig. 4C). In cells stimulated with ISO, puerarin or rapamycin pretreatment reduced cleavage of caspase-3 and diminished the number of apoptotic cells significantly. Importantly, the protective role of puerarin against apoptosis was blocked by treatment with 3-MA.

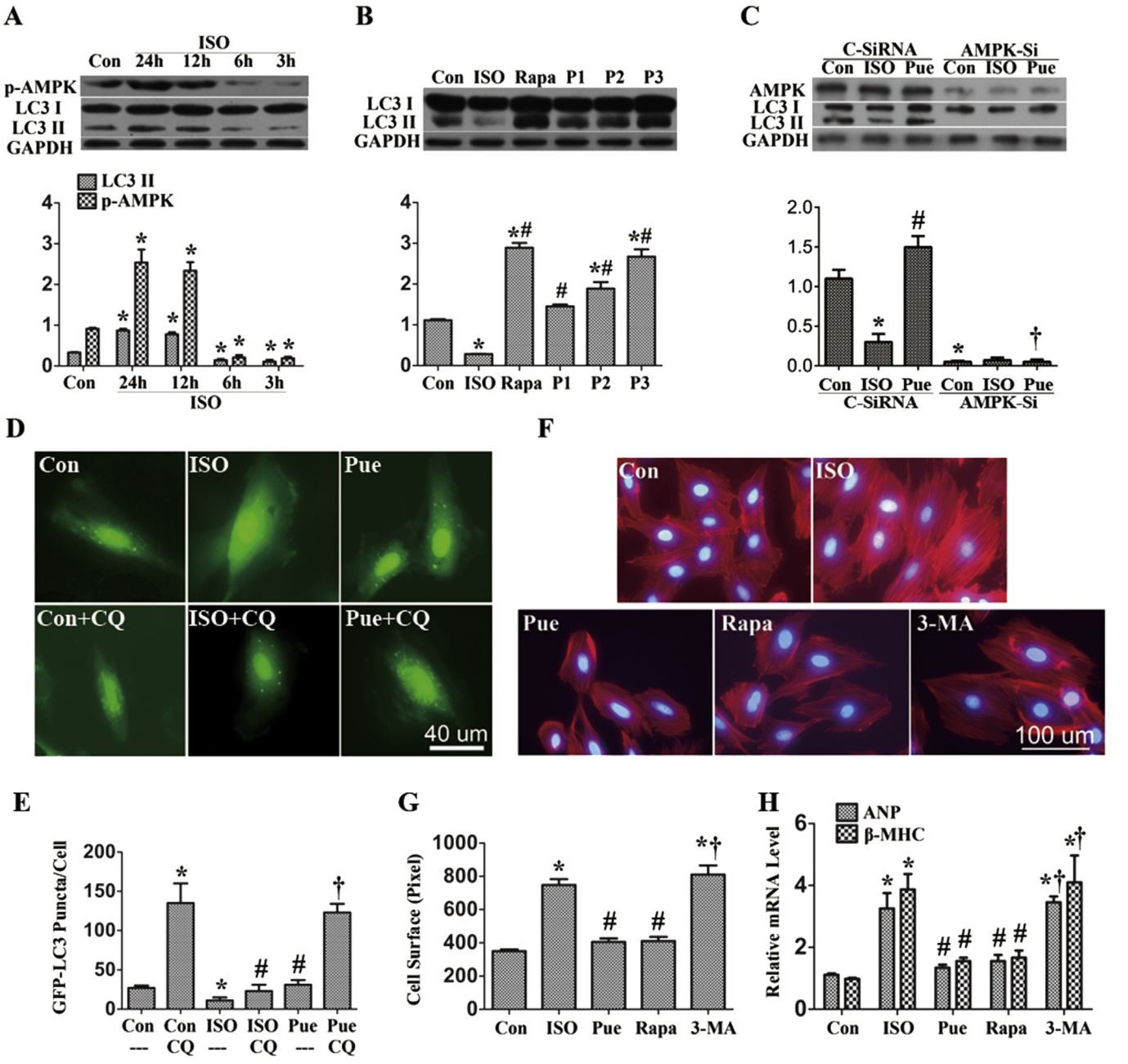

Fig. 3. Effects of puerarin on autophagy and cardiomyocyte hypertrophy. A: Effects of isoprenaline on autophagy in H9c2 with time. Representative immunoblot and quantitative analysis of the LC3B, p-AMPK, and GAPDH levels. *B*: Representative immunoblotting and quantitative analysis of LC3B and GAPDH levels; *C*: H9c2 cells were transfected with C-siRNA or AMPK-si for 48 h. The cells were subsequent treated in ISO medium with or without Puerarin. LC3B protein level was detected using immunoblotting. D and E: Representative fluorescence images and quantitative analysis showing the effect of puerarin (20 μM) on the autophagic flux. *F*: Assessment of cardiomyocyte morphology after staining with Alexa Fluor 555 phalloidin and DAPI; *G*: quantitative analysis of cardiomyocyte surface area; *H*: quantitative reverse transcription PCR analysis of the hypertrophy-related genes *ANP* and *β-MHC*. Con, control (untreated H9c2 cells); ISO, isoprenaline (1 μM) treated cells. Rapa, rapamycin (100 nM) treated cells; Pue, puerarin (20 μM) treated cells; Pue_1, puerarin 10 μM; Pue_2, puerarin 20 μM; Pue_3, puerarin 40 μM; CQ, chloroquine 10 μM pretreated cells. 3-MA, 3-methyladenine (10 mM) treated cells. $^{*}P < 0.05$ vs. control cells; $^{\#}P < 0.05$ vs. ISO-treated cells. $^{\dagger}P < 0.05$ vs. Pue or Pue/C-siRNA treated cells. Each of the experiments was repeated four times, n = 4.

3.9. Puerarin regulated mTOR signaling in cardiac hypertrophy in vivo

At week 3 after AB surgery, a significant increase in phosphorylation of mTOR and its downstream effectors S6K and 4EBP1 were observed. After puerarin or rapamycin treatment in AB rats, expression of mTOR, S6K and 4EBP1 phosphorylation was significantly decreased, indicating that an inhibitory effect of puerarin on mTOR might result in induction of autophagy (Fig. 4D).

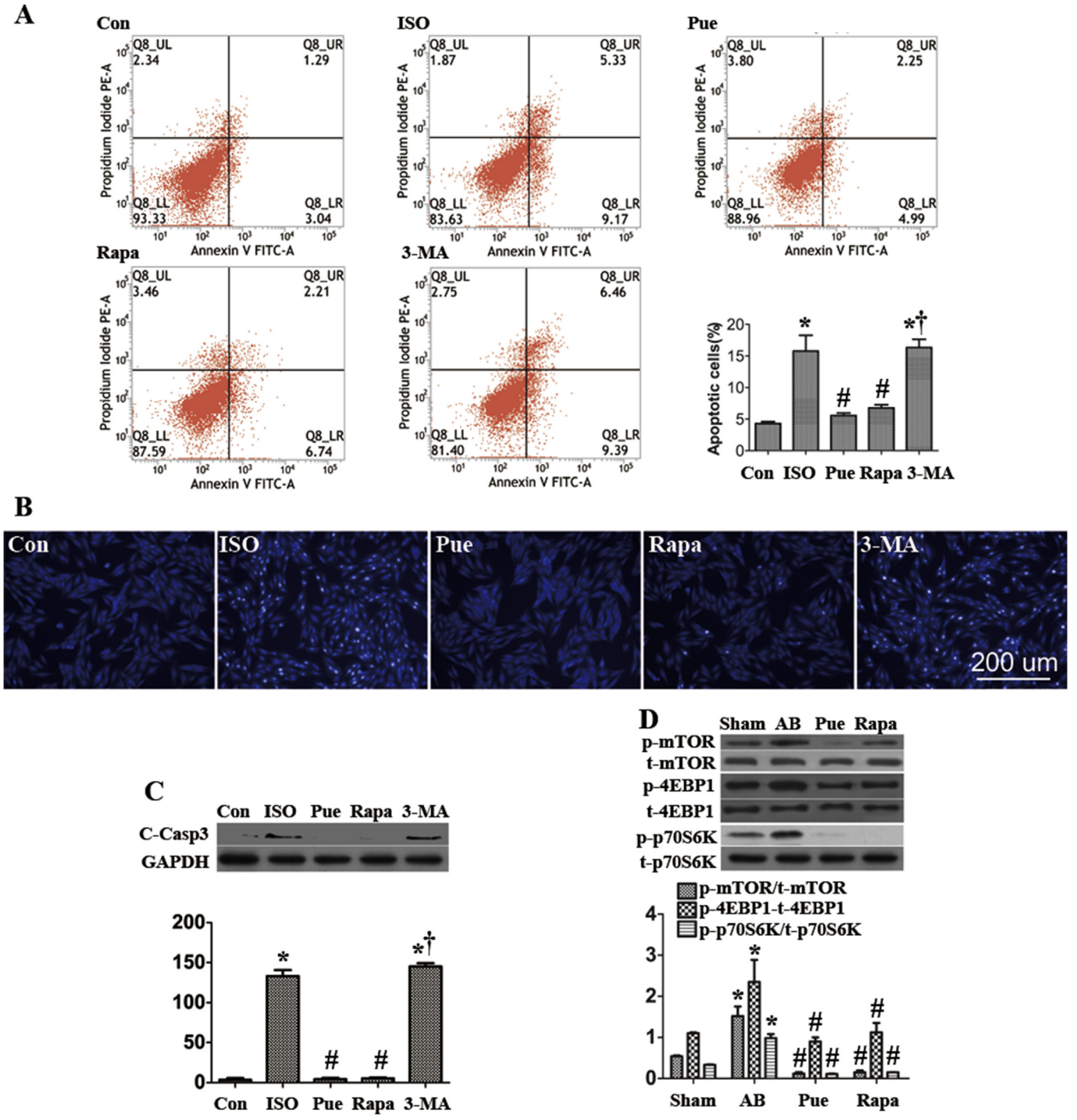

Fig. 4. Effects of puerarin on apoptosis *in vitro* and mTOR signaling *in vivo*. *A*: The apoptotic ratios of H9c2 cells were determined by flow cytometry using FITC-annexin V/PI double staining. *B*: Representative fluorescence images showing apoptotic cells stained by Hoechst 33342; *C*: Representative immunoblot and quantitative analysis of cleaved caspase 3 and GAPDH levels; *D*: Representative immunoblot and quantitative analysis of phospho-mTOR, total mTOR, 4EBP1, and p70S6K levels. Con, untreated H9c2 cells; ISO, isoprenaline (1 μM) treated cells. Rapa, rapamycin (100 nM) treated cells; Pue, puerarin (20 μM) treated cells; 3-MA, 3-methyladenine (10 mM) treated cells. AB, aortic banding group; Pue, puerarin-treated aortic banding group; Rapa, rapamycin-treated aortic band (positive control) group. $^{*}P < 0.05$ vs Control normal cells or sham group rats. $\#P < 0.05$ vs ISO treated cells or AB group rats. $\dagger P < 0.05$ vs. Puerarin treated cells. Each of the experiments was repeated four times, n = 4.

4. Discussion

The results of the present study are summarized as follows: (1) puerarin could attenuate the progress of cardiac hypertrophy and inhibit cardiomyocyte apoptosis; (2) the protective effects of puerarin against cardiac hypertrophy and apoptosis might be exerted partly through restoration of autophagy, and (3) regulation of AMPK/mTOR signaling might be involved in this puerarin-induced activation of autophagy.

Consistent with previous data [10–14], present study revealed that AB rats treated with puerarin for 6 weeks showed no significant difference from SO rats in terms of indicators for either hypertrophy or apoptosis. Similarly, puerarin treatment was also able to decrease cell size, expression of ANP and β-MHC mRNA, and apoptosis in ISO-stimulated H9c2 cells. Autophagy has been shown to play an important role in the reaction of cardiomyocytes to numerous types of stress. Previous data from animal model of cardiac hypertrophy have indicated that basal autophagy is pivotal in the maintenance of myocardial cell morphology and cardiac function through elimination of injured organelles and proteins, whereas excessive activated autophagy has been shown to be able to induce cell death. In the early stage of cardiac hypertrophy decreased autophagy can be observed, and subsequently promotes further exacerbation of the condition. Moreover, inhibition of autophagy can also be detected in a hypertrophic model of animals subjected to AB. The specific deficiency of ATG5 causing cardiac hypertrophy and dysfunction further offers evidence of the protective role of basal autophagy in the development of cardiac hypertrophy [5]. However, in cases of sustained overload stimulation, heart failure occurs, and this in turn activates autophagy, consequently leading to myocardial death. Thus, the changes in autophagic level might be an indicator reflecting different stages of cardiac hypertrophy [2,5,15], and the duration of decreased autophagy in the period of compensated hypertrophy relies on the variable extents of hemodynamic stress. In order to guarantee a sufficient duration of compensated hypertrophy for detecting decreased autophagy, we performed descending AB according to the description by Li et al. [2] to achieve a moderate pressure overload in rats.

As an activator of autophagy, rapamycin has been shown to be able to prevent cardiac hypertrophy in animals subjected to thyroid hormone or isoprenaline treatment, and even to reverse stress-induced cardiac hypertrophy, leading to improved cardiac function [16,17]. Similar to previous data, the results of the present study using rapamycin treatment as a positive control in AB rats showed reproducible protective effects, including prevention of cardiac hypertrophy progression, retardation of remodeling, and inhibition of cardiomyocyte apoptosis, accompanied by activation of autophagy. Intriguingly, puerarin treatment produced similar effects of autophagy activation and cardiac protection as those seen with rapamycin. After 3-MA pretreatment of H9c2 of cells, co-incubation of puerarin and ISO failed to produce reductions in indicators of both hypertrophy and apoptosis *in vitro* study. These results indicated that the protective impact of puerarin might be partly autophagy-dependent.

To date, there are limited studies focusing on the underlying mechanism of puerarin in regulating autophagy. In the Parkinson's disease, treatment with puerarin was shown to protect against increased level of reactive oxygen species, and prevent oxidative injury through the chaperone-mediated autophagy pathway [18]. In ethanol-treated hepatocytes, incubation with puerarin was able to significantly recover cell viability and to reduce cellular lipid accumulation through activation of autophagy. Further investigation revealed that regulation of AMPK/mTOR signaling might be involved in the molecular mechanisms [19]. Importantly, accumulated data have demonstrated that AMPK/mTOR signaling is also involved in the development of cardiac hypertrophy. Inactivated AMPK can be observed in the early stage of compensated cardiac hypertrophy *in vivo* or during the first hours of hypertrophic stimulation of H9c2 cells [2]. In AMPK alpha-2 null mice, the cardiac hypertrophy induced by isoproterenol or aortic constriction was significantly greater than in controls [20]. Furthermore, the cardiospecific deletion of LKB1 was shown to lead to hypertrophy, correlated with stimulation of mTOR signaling and reduced AMPK phosphorylation, and could be prevented by overexpressing a constitutively active form of AMPK or by inhibiting mTOR with rapamycin [21,22]. In our *in vivo* study, decreased levels of phosphorylated AMPK were observed, accompanied by reduced LC3II. Similar phenomena were also detected within 6 h *in vitro* by ISO treatment of H9c2 cells. Naturally, AMPK has been considered as a therapeutic target in the management of cardiac hypertrophy and other metabolic diseases such as diabetes mellitus and ischemic heart disease. Applying AMPK activators such as 5-aminoimidazole-4-carboxamide ribonucleotide or metformin has been confirmed to activate autophagy and to exert anti-hypertrophy or cardioprotective effects [2,23].

Inhibition of mTOR by its specific inhibitor rapamycin or partial ablation of mTOR blocked p70S6K activation and counteracted the development of cardiac hypertrophy. In present study, pharmacological activation of AMPK using puerarin inhibited the mTOR pathway, as confirmed by significantly reduced S6 phosphorylation and 4E-BP1 phosphorylation in puerarin-treated AB rats compared with untreated AB rats. This suggests an inhibitory effect of puerarin on mTOR that results in the induction of autophagy. Together, these results imply that puerarin attenuates the development of hypertrophy by increasing autophagy, partly through stimulating AMPK phosphorylation, and subsequently suppresses mTOR phosphorylation.

Conflict of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.07.065.

Transparency document

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References

[1] P. Wojciechowski, D. Juric, X.L. Louis, S.J. Thandapilly, L. Yu, C. Taylor, T. Netticadan, Resveratrol arrests and regresses the development of pressure overload- but not volume overload-induced cardiac hypertrophy in rats, J. Nutr. 140 (2010) 962–968.
[2] Y. Li, C. Chen, F. Yao, Q. Su, D. Liu, R. Xue, G. Dai, R. Fang, J. Zeng, Y. Chen, H. Huang, Y. Ma, W. Li, L. Zhang, C. Liu, Y. Dong, AMPK inhibits cardiac hypertrophy by promoting autophagy via mTORC1, Arch. Biochem. Biophys. 15 (2014) 79–86.
[3] N. Mizushima, M. Komatsu, Autophagy: renovation of cells and tissues, Cell 147 (2011) 728–741.

[4] B. Levine, G. Kroemer, Autophagy in the pathogenesis of disease, Cell 132 (2008) 27–42.
[5] A. Nakai, O. Yamaguchi, T. Takeda, Y. Higuchi, S. Hikoso, M. Taniike, S. Omiya, I. Mizote, Y. Matsumura, M. Asahi, K. Nishida, M. Hori, N. Mizushima, K. Otsu, The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress, Nat. Med. 13 (2007) 619–624.
[6] C. Liu, R. Xue, D. Wu, L. Wu, C. Chen, W. Tan, Y. Chen, Y. Dong, REDD1 attenuates cardiac hypertrophy via enhancing autophagy, Biochem. Biophys. Res. Commun. 454 (2014) 215–220.
[7] H.L. Chen, X. Wang, M.M. Tong, D. Wu, S.S. Wu, J.X. Chen, X.X. Wang, X.L. Wang, Y. Kang, H. Tang, C.S. Tang, W. Jiang, Intermedin Suppresses Pressure Overload Cardiac Hypertrophy through Activation of Autophagy, PLoS One 8 (2013) e64757.
[8] Y.X. Zhou, H. Zhang, C. Peng, Puerarin: A Review of Pharmacological Effects, Phytother. Res. 28 (2014) 961–975.
[9] J. Li, R. Liu, Y. Lei, K. Wang, Q.C. Lau, N. Xie, S. Zhou, C. Nie, L. Chen, Y. Wei, C. Huang, Proteomic analysis revealed association of aberrant ROS signaling with suberoylanilide hydroxamic acidinduced autophagy in Jurkat T-leukemia cells, Autophagy 6 (2010) 711–724.
[10] H. He, M. Shi, J. Yang, X. Zeng, H. Qiao, L. Wu, L. Li, The correlation between angiogenesis and abnormal expression of SERCA2a, phosphorlamban and the endothelin pathway in heart failure, and improvement by puerarin, Phytother. Res. 22 (2008) 948–956.
[11] G. Chen, S.Q. Pan, C. Shen, S.F. Pan, X.M. Zhang, Q.Y. He, Puerarin inhibits angiotensin II-induced cardiac hypertrophy via the redox-sensitive ERK1/2, p38 and NF-κB pathways, Acta. Pharmacol. Sin. 35 (2014) 463–475.
[12] R. Chen, J. Xue, M. Xie, Puerarin prevents isoprenaline-induced myocardial fibrosis in mice by reduction of myocardial TGF-β1 expression, J. Nutr. Biochem. 23 (2012) 1080–1085.
[13] Y. Yuan, J. Zong, H. Zhou, Z.Y. Bian, W. Deng, J. Dai, H.W. Gan, Z. Yang, H. Li, Q.Z. Tang, Puerarin attenuates pressure overload-induced cardiac hypertrophy, J. Cardiol. 63 (2014) 73–81.
[14] B.A. Rothermel, J.A. Hill, Autophagy in Load-Induced Heart Disease, Circ. Res. 103 (2008) 1363–1369.
[15] H. Zhu, P. Tannous, J.L. Johnstone, Y. Kong, J.M. Shelton, J.A. Richardson, V. Le, B. Levine, B.A. Rothermel, J.A. Hill, Cardiac autophagy is a maladaptive response to hemodynamic stress, J. Clin. Invest 117 (2007) 1782–1793.
[16] J.A. Kuzman, T.D. O'Connell, A.M. Gerdes, Rapamycin prevents thyroid hormone-induced cardiac hypertrophy, Endocrinology 148 (2007) 3477–3484.
[17] X. Chen, S.Y. Zeng, J. Zou, Y.F. Chen, Z.B. Yue, Y. Gao, L.K. Zhang, W.W. Cao, P.Q. Liu, Rapamycin attenuated cardiac hypertrophy induced by isoproterenol and maintained energy homeostasis via inhibiting NF-κB activation, Mediat. Inflamm. 2014 (2014) 868753.
[18] X.C. Wang, X. Wang, Q.L. Li, Effect of chaperone-mediated autophagy in MPP(+) -induced SH-SY5Y cells and interventional effect of puerarin, Zhong Guo Zhong Yao Za Zhi 39 (2014) 106–112.
[19] B.K. Noh, J.K. Lee, H.J. Jun, J.H. Lee, Y.Y. Jia, M.H. Hoang, J.W. Kim, K.H. Park, S.J. Lee, Restoration of autophagy by puerarin in ethanol-treated hepatocytes via the activation of AMP-activated protein kinase, Biochem. Biophys. Res. Commun. 414 (2011) 361–366.
[20] P. Zhang, X. Hu, X. Xu, J. Fassett, G. Zhu, B. Viollet, W. Xu, B. Wiczer, D.A. Bernlohr, R.J. Bache, Y. Chen, AMP activated protein kinase-alpha2 deficiency exacerbates pressure-overload-induced left ventricular hypertrophy and dysfunction in mice, Hypertension 52 (2008) 918–924.
[21] Y. Ikeda, K. Sato, D.R. Pimentel, F. Sam, R.J. Shaw, J.R. Dyck, K. Walsh, Cardiac-specific deletion of LKB1 leads to hypertrophy and dysfunction, J. Biol. Chem. 284 (2009) 35839–35849.
[22] N. Jessen, H.J. Koh, C.D. Folmes, C. Wagg, N. Fujii, B. Løfgren, C.M. Wolf, C.I. Berul, M.F. Hirshman, G.D. Lopaschuk, L.J. Goodyear, Ablation of LKB1 in the heart leads to energy deprivation and impaired cardiac function, Biochim. Biophys. Acta 1802 (2010) 593–600.
[23] C.Y. He, H.P. Zhu, H.L. Li, M.H. Zou, Z.L. Xie, Dissociation of Bcl-2–Beclin1 Complex by Activated AMPK Enhances Cardiac Autophagy and Protects Against Cardiomyocyte Apoptosis in Diabetes, Diabetes 62 (2013) 1270–1281.