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AMPK regulates energy metabolism through the SIRT1 signaling pathway to improve myocardial hypertrophy

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Abstract. – OBJECTIVE: We investigated the correlations of adenosine monophosphate-activated protein kinase (AMPK), Silence information regulator 1 (SIRT1) and energy metabolism with myocardial hypertrophy.

MATERIALS AND METHODS: Myocardial hypertrophy experimental model was established via transverse aortic constriction (TAC)-induced myocardial hypertrophy and phenylephrine (PE)-induced hypertrophic myocardial cell culture. After activation of AMPK, the messenger ribonucleic acid (mRNA) expressions in myocardial tissue- and myocardial cell hypertrophy-related genes, atrial natriuretic peptide (ANP) and β-myosin heavy chain (β-MHC), were detected. The production rate of ^{14}C-labeled $^{14}CO_2$ from palmitic acid was quantitatively determined to detect the fatty acid and glucose oxidation of hypertrophic myocardial tissues or cells, and the glucose uptake of myocardial cells was studied using [^{14}C] glucose. Reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting were performed to detect the changes in SIRT1 mRNA and protein expressions in hypertrophic myocardial tissues. Moreover, SIRT1 small interfering ribonucleic acid (siRNA) was used to interfere in SIRT1 expression to further investigate the role of SIRT1 in the effect of AMPK activation on myocardial hypertrophy.

RESULTS: AMPK activation could significantly reduce the mRNA expressions of ANP and β-MHC *in vitro* and *in vivo*. AMPK could increase the ejection fraction (EF) and decrease the protein synthesis rate in myocardial cells in mice with myocardial hypertrophy. Besides, AMPK activation could increase the fatty acid oxidation, improve the glucose uptake and reduce the glucose oxidation. After AMPK activation, both SIRT1 mRNA and protein expressions in hypertrophic myocardial tissues and myocardial cells were increased. After SIRT1 siRNA was further used to interfere in SIRT1 expression in myocardial cells, it was found that mRNA expressions and protein synthesis rates of ANP and β-MHC were increased.

CONCLUSIONS: The activation of AMPK can inhibit the myocardial hypertrophy, which may be realized through regulating the myocardial energy metabolism via SIRT1 signaling pathway.

Key Words:
Myocardial hypertrophy, AMPK, SIRT1, Energy metabolism.

Introduction

Myocardial hypertrophy is an adaptive response of the heart to many exogenous stimuli, such as hypertension, valvular heart disease, myocardial infarction and cardiomyopathy. It will lead to heart failure, arrhythmia and even sudden death in the long term, although this process is a compensatory response to the increased cardiac work[1]. To date, heart failure is the most common complication of myocardial hypertrophy, as well as one of the leading causes of human death[2]. At present, the drug therapy of heart failure is actualized mainly through changing the neuroendocrine to improve the cardiac systolic function or correct myocardial calcium transport. Although these programs can improve the cardiac function in a short term, the 5-year mortality rate of patients with heart failure is still close to 50%[3]. Therefore, it is necessary to search new treatment methods and better understand the occurrence mechanism of myocardial hypertrophy. Myocardial hypertrophy is characterized by increases in the volume of myocardial cell volume, protein synthesis and number of muscle fibers. Currently, the occurrence mechanism of myocardial hypertrophy is not fully clear yet. The energy metabolic disturbance of myocardial cells, decreased fatty acid oxidation and increased glucose utilization,

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are important causes of myocardial hypertrophy[4-6]. Thus, exploring the mechanism of energy metabolic disturbance of myocardial cells is of great significance in searching new target for the treatment of myocardial hypertrophy.

Adenosine monophosphate-activated protein kinase (AMPK) is a kind of serine/threonine protein kinase, widely existing in eukaryotic organisms, and its major role in myocardium is to regulate the energy metabolism[7,8]. The activation of AMPK is beneficial to the heart through the activation of energy-producing pathways and inhibition of energy expenditure. AMPK can regulate the fatty acid metabolism via promoting the fatty acid uptake, transport and oxidation; moreover, it can regulate the glucose metabolism through promoting glucose uptake and glycolysis and inhibiting glycogen synthesis. Therefore, AMPK is a key regulator of myocardial energy metabolism, but whether it affects myocardial hypertrophy is not fully understood yet. Silent information regulator 1 (SIRT1), a member of the sirtuins family, is the histone deacetylase that is dependent on nicotinamide adenine dinucleotide (NAD+). In recent years, researches have shown that SIRT1 can promote the glucose and lipid metabolism[8,9], and participate in the pathophysiological processes of atherosclerosis[10], myocardial ischemia[11], myocardial hypertrophy[12] and other cardiovascular diseases. Existing studies[13,14] have shown that AMPK can enhance the SIRT1 activity by up-regulating the NAD+ level in cells. Therefore, AMPK and SIRT1 are thought to be jointly involved in the cellular energy metabolism process.

We analyzed the occurrence mechanism of myocardial hypertrophy. AMPK/SIRT1/energy metabolism pathways were selected as starting points for the study. Myocardial hypertrophy experimental model was established via aortic coarctation-induced myocardial hypertrophy and phenylephrine (PE)-induced hypertrophic myocardial cell culture to study the correlations of AMPK, SIRT1 and energy metabolism with myocardial hypertrophy.

Materials and Methods

Experimental Animals and Models

Male inbred Sprague-Dawley (SD) rats, aged 14-16 weeks old, weighing 250-270 g, were provided by Liaocheng People's Hospital Laboratory Animal Center. This study was approved by the Animal Ethics Committee of Liaocheng People's Hospital Animal Center. Transverse aortic constriction (TAC) model[15] was as follows: after rats were anesthetized via intraperitoneal injection of 4% pentobarbital sodium solution (40 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA), they were fixed under a supine position; a 2 cm-long longitudinal incision was made below the xiphoid in the center of abdomen, and the abdominal aorta was explored in the abdominal cavity; a small segment of abdominal aorta was separated at about 3 mm above the branch point of right renal artery from the abdominal aorta, and then a syringe needle with a diameter of 0.8 mm and this segment of abdominal aorta were ligatured together using a No. 4 suture; after that, the needle was quickly withdrawn, causing the inner diameter constriction of abdominal aorta in ligation site.

Myocardial Cell Extraction and Culture

The chest of newborn clean-grade SD rat aged 1-3 days old was opened to take the heart. Ventricular tissues were separated and cut into tissue blocks (about 1 mm^3). 0.1% trypsin (Thermo Fisher Scientific, Waltham, MA, USA) was added for digestion in a thermostatic water bath box at 37°C for about 5 min, then 0.05% type I collagenase (Thermo Fisher Scientific, Waltham, MA, USA) was added in a volume ratio of 1:10, followed by digestion in the thermostatic water bath box at 37°C for 3-4 h. The centrifuge tube was taken out, the upper-layer suspension was discarded and 0.1% trypsin was added for digestion in the thermostatic water bath box at 37°C for 7-8 min. The upper-layer suspension was taken and transferred into another centrifuge tube. Dulbecco's modified Eagle medium: nutrient mixture F-12 (DMEM/F12) (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) was added to terminate the digestion. The remaining sediment was added with 0.1% trypsin; the above steps were repeated for 2-3 times. The suspension was centrifuged for 10 min, and the sediment was diluted with DMEM/F12 containing 10% fetal bovine serum, mixed evenly and filtered using a screen mesh. The resulting solution was cultured in a culture flask for 1 h to remove fibroblasts. After 1 h, the flask was shaken, and the suspension was taken and counted. Then, it was inoculated at a density of $1\times10^5/cm^2$, and incubated in a thermostatic incubator with 5% CO_2 at 37°C. The solution was replaced on the next day, and added with bro-

modeoxyuridine (BrdU) (Thermo Fisher Scientific, Waltham, MA, USA) to inhibit the fibroblast growth. On the third day, a variety of intervention experiments could be performed.

Ribonucleic Acid (RNA) Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Myocardial tissues or cells were added with TRIzol cell lysis buffer (Invitrogen, Carlsbad, CA, USA), and the homogenate was collected into an Eppendorf (EP) tube. The chloroform was added, and the tube was shaken violently for 15 s, followed by centrifugation for 15 min. The supernatant was taken, added with isopropanol, let stand at room temperature for 10 min and centrifuged, and the supernatant was discarded. 75% ethanol prepared by diethylpyrocarbonate (DEPC) (Sigma-Aldrich, St. Louis, MO, USA)-treated water was added for washing, followed by centrifugation for 15 min. The supernatant was discarded, and the solution was air-dried at room temperature for 10-15 min, and added with sterilized DEPC-treated water. The content and purity of the resulting RNA aqueous solution were determined. Primers were synthesized according to target genes, and PCR was performed after complementary deoxyribonucleic acid (cDNA) was obtained via reverse transcription.

Protein Extraction and Western Blotting

An appropriate amount of lysis solution was added into the myocardial tissues or cells, and the tissue homogenate or cell lysis buffer was centrifuged. The supernatant was taken. After protein quantification, the loading buffer was added, and the mixture was boiled at 95°C for 5 min for stand-by application. After electrophoresis under constant voltage, the protein was transferred onto a polyvinylidene difluoride (PVDF) membrane, sealed at room temperature and incubated using the primary antibody of target protein at 4°C overnight. On the next day, the secondary antibody and the developing solution were added for incubation, followed by exposure and photography.

Cell Transfection

Myocardial cells of rats were inoculated into a 6-well plate (5×10^5/well). On the third day of culture, the serum-free DMEM was used, and cells were cultured for 2 h. SIRT1 small interfering ribonucleic acid (siRNA) (Hanbio Biotechnology, Shanghai, China) and lipidosome were mixed and added into serum-free DMEM. The mixture was shaken gently and incubated at room temperature for 15 min. 400 μL Mixture were added into each well, and incubated with 5% CO_2 for 6 h; then, the serum-free medium was replaced with medium containing 10% fetal bovine serum (FBS). After 48 h, the total protein was extracted and analyzed via Western blotting to understand the inhibitory effect of SIRT1.

Detection of fatty Acid and Glucose Oxidation

The production rate of ^{14}C-labeled $^{14}CO_2$ from palmitic acid was quantitatively determined to detect the fatty acid and glucose oxidation. The heart was perfused with KH perfusate containing 1.0 mM [^{14}C] palmitic acid (Seebio Biotech, Shanghai, China) for 1 h. The $^{14}CO_2$ released and $^{14}CO_2$ in the perfusate were collected, forming [^{14}C] sodium bicarbonate with NaOH in the filter paper. The filter paper was dried to count [^{14}C] sodium bicarbonate in a scintillation flask.

Glucose Uptake

To determine the glucose uptake of myocardial tissues, the heart was perfused with [^{14}C] glucose to detect its absorptive amount. After cardiac perfusion for 60 min, [^{14}C] glucose perfusate was collected to analyze the amount of [^{14}C] glucose in the perfusate. [^{14}C] glucose activity was detected in the scintillation flask. The difference in radioactive counting between the initial and final samples indicated the amount of [^{14}C] glucose taken by the heart.

[^{3}H] Leucine Incorporation

After interference with myocardial cells inoculated, 1 μ Ci [^{3}H] leucine (Seebio, Shanghai, China) was added into each well. After 48 h of culture, cells were collected and fixed using 10% trichloroacetic acid for 30 min. NaOH was added into each well to dissolve cells. 100 μL cells were pipetted from each well onto a glass fiber filter membrane; after being dried, they were placed into the scintillation flask and added with 3 mL scintillation fluid. The incorporation amount of [^{3}H] leucine was determined using a LS-6500 liquid scintillation counter.

Statistical Analysis

All experimental results were presented as mean ± standard deviation ($\bar{x} \pm s$). Student's *t*-test was used for the pairwise comparison, and one-way analysis of variance (ANOVA) was used for

the intergroup comparison, followed by Least Significant Difference (LSD). Statistical product and service solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) were used for all statistics. $p < 0.05$ suggested that the difference was statistically significant.

Results

Inhibitory Effect of AMPK Activation on Myocardial Hypertrophy

In-vivo experiments: compared with those in sham-operation (Sham) group, messenger ribonucleic acid (mRNA) expression levels of ANP and β-MHC in Acadesine (AICAR) intervention + Sham (Sham + AI) group were not influenced. The mRNA expression levels of ANP and β-MHC in TAC model (TAC) group were significantly higher than those in Sham group. The mRNA expressions of ANP and β-MHC in AI intervention + TAC (TAC + AI) group were significantly decreased compared with those in TAC group (Figure 1A-B). EF slope was not affected in Sham + AI group compared with that in Sham group. EF slope in TAC group was significantly lower than that in Sham group, while it was significantly higher in TAC + AI group than that in model group (Figure 1C). *In-vitro* experiments: mRNA expression levels of ANP and β-MHC in simple AI intervention (AI) group were not influenced compared with those in normal control (CON) group. The mRNA expressions of ANP and β-MHC in PE intervention (PE) group were higher than those in CON group; the mRNA expressions of ANP and β-MHC in AI + PE intervention (PE + AI) group were significantly decreased compared with those in PE group. The mRNA expressions of ANP and β-MHC in Compound C + PE (PE + CC) group were further increased compared with those in PE group (Figure 1D-E). The incorporation amount of [^{3}H] leucine in AI group was not affected compared with that in CON group. The incorporation amount of [^{3}H] leucine in PE group was higher than that in CON group; the incorporation amount of [^{3}H] leucine in PE + AI group was decreased compared with that in PE group, while it was increased in PE + CC group compared with that in PE group (Figure 1F).

Effect of AMPK on Fatty Acid Metabolism in Hypertrophic Myocardium

In-vivo experiments: [^{14}C] palmitic acid oxidation was not affected in Sham + AI group compared with that in Sham group. [^{14}C] palmitic acid oxidation amount in TAC group was lower than that in Sham group, while it was increased significantly in TAC + AI group compared with that in TAC group (Figure 2A). *In-vitro* experiments:

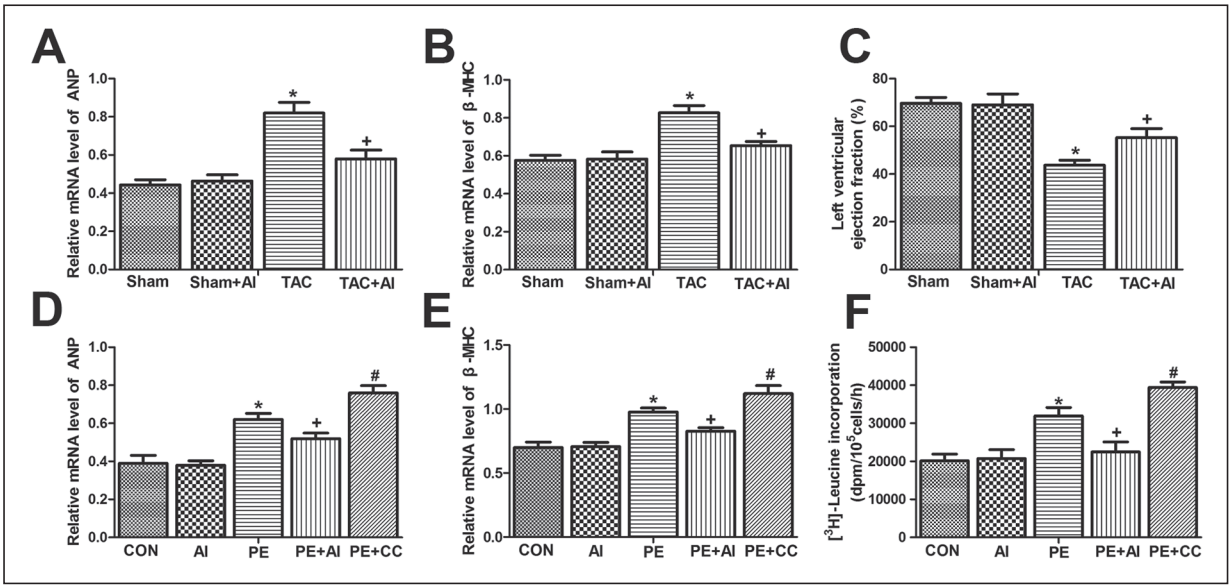

Figure 1. Inhibitory effect of AMPK activation on myocardial hypertrophy. ***A,*** Analysis of mRNA level of ANP *in vivo*. ***B,*** Analysis of mRNA level of β-MHC *in vivo*. ***C,*** Analysis of left ventricular ejection fraction (LVEF). ***D,*** Analysis of mRNA level of ANP *in vitro*. ***E,*** Analysis of mRNA level of β-MHC *in vitro*. ***F,*** Analysis of [^{3}H] leucine incorporation *in vitro*. *$p <$ 0.05 *vs.* Sham group or CON group, + $p < 0.05$ *vs.* TAC group or PE group, $^{\#}$*vs.* PE group.

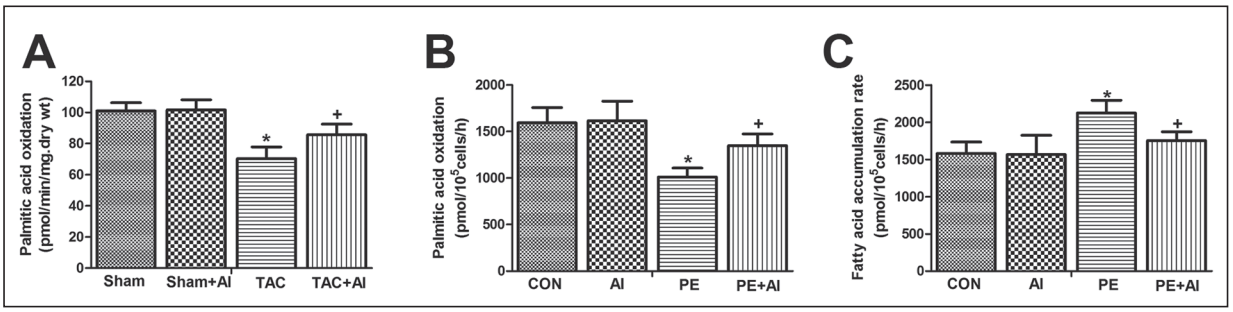

Figure 2. Effect of AMPK on fatty acid metabolism in hypertrophic myocardium. ***A,*** Analysis of palmitic acid oxidation *in vivo*. ***B,*** Analysis of palmitic acid oxidation *in vitro*. ***C,*** Analysis of fatty acid deposition rate *in vitro*. **p* < 0.05 *vs.* Sham group or CON group, + *p* < 0.05 *vs.* TAC group or PE group.

[^{14}C] palmitic acid oxidation rate was not affected in AI group compared with that in CON group. [^{14}C] palmitic acid oxidation rate in PE group was lower than that in CON group, while it was significantly increased in PE + AI group compared with that in PE group. [^{14}C] palmitic acid deposition rate was not affected in AI group compared with that in CON group. [^{14}C] palmitic acid deposition rate in PE group was higher than that in CON group, while it was decreased in PE + AI group compared with that in PE group (Figure 2B-C).

Effect of AMPK on Glucose Metabolism in Hypertrophic Myocardium

In-vivo experiments: [^{14}C] glucose oxidation was not affected in Sham + AI group compared

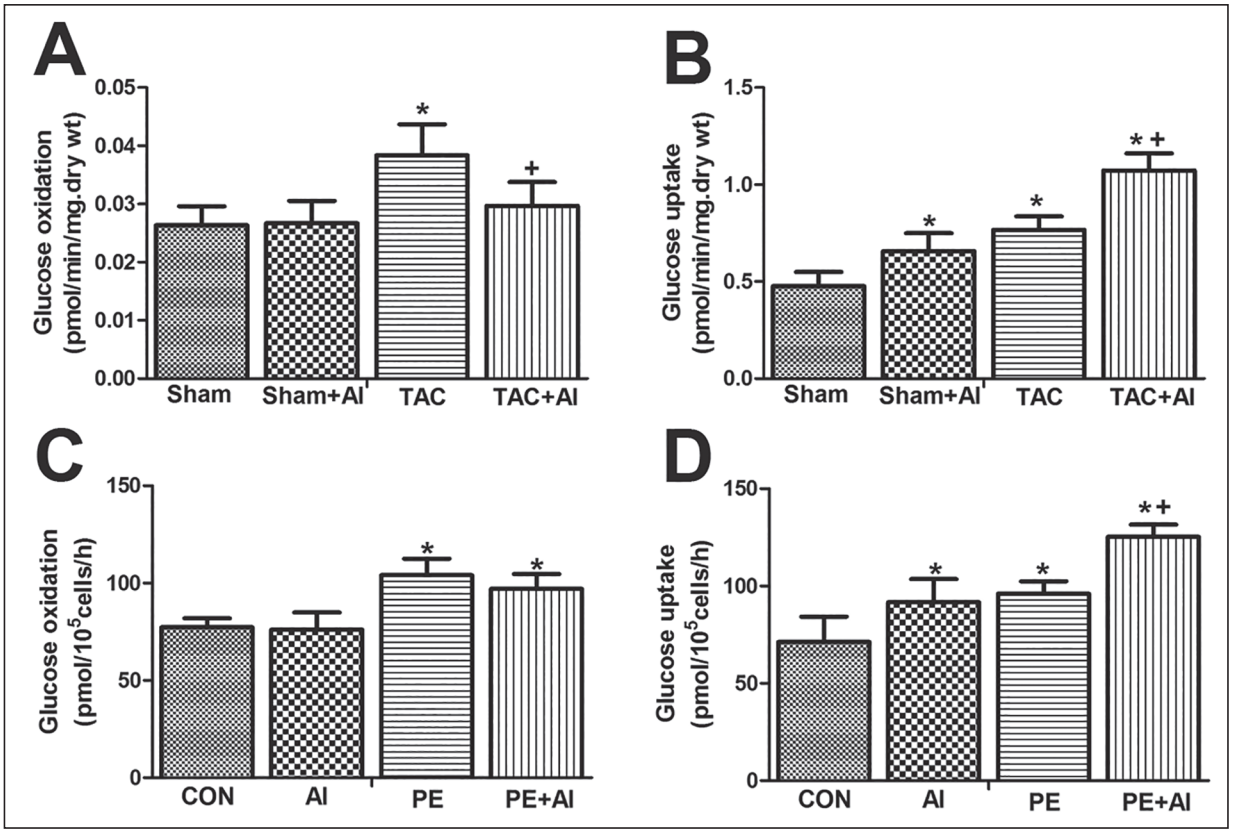

Figure 3. Effect of AMPK on glucose metabolism in hypertrophic myocardium. ***A,*** Analysis of glucose oxidation *in vivo*. ***B,*** Analysis of glucose uptake *in vivo*. ***C,*** Analysis of glucose oxidation in *in vitro*. ***D,*** Analysis of glucose uptake *in vitro*. **p* < 0.05 *vs.* Sham group or CON group, + *p* < 0.05 *vs.* TAC group or PE group.

with that in Sham group. [^{14}C] glucose oxidation amount in TAC group was significantly increased compared with that in Sham group, while it was decreased in TAC + AI group compared with that in TAC group. The glucose uptake amount in Sham + AI group was increased compared with that in Sham group; [^{14}C] glucose uptake amount in TAC group was increased compared with that in Sham group, and it was also significantly increased in TAC + AI group compared with that in TAC group [^{14}C] (Figure 3A-B).

In-vitro experiments: [^{14}C] glucose oxidation in AI group was not affected compared with that in CON group. [^{14}C] glucose oxidation in PE group was higher than that in CON group, and it was decreased slightly in PE + AI group compared with that in PE group. [^{14}C] glucose uptake rate was not affected in AI group compared with that in CON group. [^{14}C] glucose uptake rate in PE group was higher than that in CON group; [^{14}C] glucose uptake rate was increased in PE + AI group compared with that in CON group, and it was also increased in PE + AI group compared with that in PE group (Figure 3C-D).

Role of SIRT1 in Improvement of Myocardial Hypertrophy Via AMPK Activation

In-vivo experiments: mRNA and protein expression levels of SIRT1 in Sham + AI group were not affected compared with those in Sham group. mRNA and protein expressions of SIRT1 in TAC group were lower than those in Sham group, while they were significantly increased in TAC + AI group compared with those in TAC group (Figure 4A-B).

In-vitro experiments: mRNA and protein expression levels of SIRT1 in AI group were not affected compared with those in CON group. The mRNA and protein expressions of SIRT1 in PE group were lower than those in normal control group, they were increased in PE + AI group compared with those in PE group, and they were further decreased in PE + CC group compared with those in PE group (Figure 4C-D). The mRNA levels of ANP and β-MHC in AI + PE + SIRT1 siRNA (PE + AI + Ss) group were significantly increased compared with those in PE + AI group, but there were no significant differences in mRNA expressions of ANP and β-MHC compared with those in PE group (Figure 5A-B). The incorporation amount of [^{3}H] leucine in PE + AI + Ss group was significantly increased compared with that in PE + AI group, but there was no significant difference in the incorporation amount of [^{3}H] leucine compared with that in PE group (Figure 5C). [^{14}C] palmitic acid oxidation rate in PE + AI + Ss group was decreased compared with that in PE + AI group, but there was no significant difference in [^{14}C] palmitic acid oxidation rate compared with that in PE group (Figure 5D). [^{14}C] palmitic acid deposition rate in PE + AI + Ss group was increased compared with that in PE + AI group, but there was no significant difference in [^{14}C] palmitic acid deposition rate compared with that in PE group (Figure 5E). [^{14}C] glucose uptake rate in PE + AI + Ss group was significantly increased compared with that in PE+AI group, and it was also increased compared with that in PE group (Figure 5F). [^{14}C] glucose oxidation in PE + AI + Ss group had no significant change compared with that in PE group, and it also had no significant difference from that in PE + AI group (Figure 5G).

Discussion

In recent years, a large number of researches have shown that AMPK activation regulates not only myocardial energy metabolism, but also hypertrophic myocardium growth. In this experiment, AMPK activator AICAR was used to interfere in TAC rat model for 8 weeks, and then the expressions of hypertrophy-related genes (ANP and β-MHC) were detected. Results showed that AICAR inhibited myocardial hypertrophy and mRNA expressions of ANP and β-MHC. The cardiac function in AI group was significantly improved compared with that in simple TAC group, indicating that AMPK activation can inhibit myocardial hypertrophy from regulating hypertrophy-related genes. In addition, the results also showed that AMPK activation improved the cardiac function through inhibiting myocardial hypertrophy. To further confirm the inhibitory effect of AMPK on myocardial hypertrophy, AMPK activator was used to interfere in PE-induced cardiomyocyte hypertrophy of neonatal rats. Results showed that AI could inhibit the mRNA expressions of hypertrophy-related genes (ANP and β-MHC) and hypertrophy-related protein synthesis. *In-vitro* experiments suggested that AMPK activation plays an important role in the formation process of myocardial hypertrophy, and activation of AMPK can inhibit the formation of myocardial hypertrophy. To further confirm the inhibitory effect of AMPK on

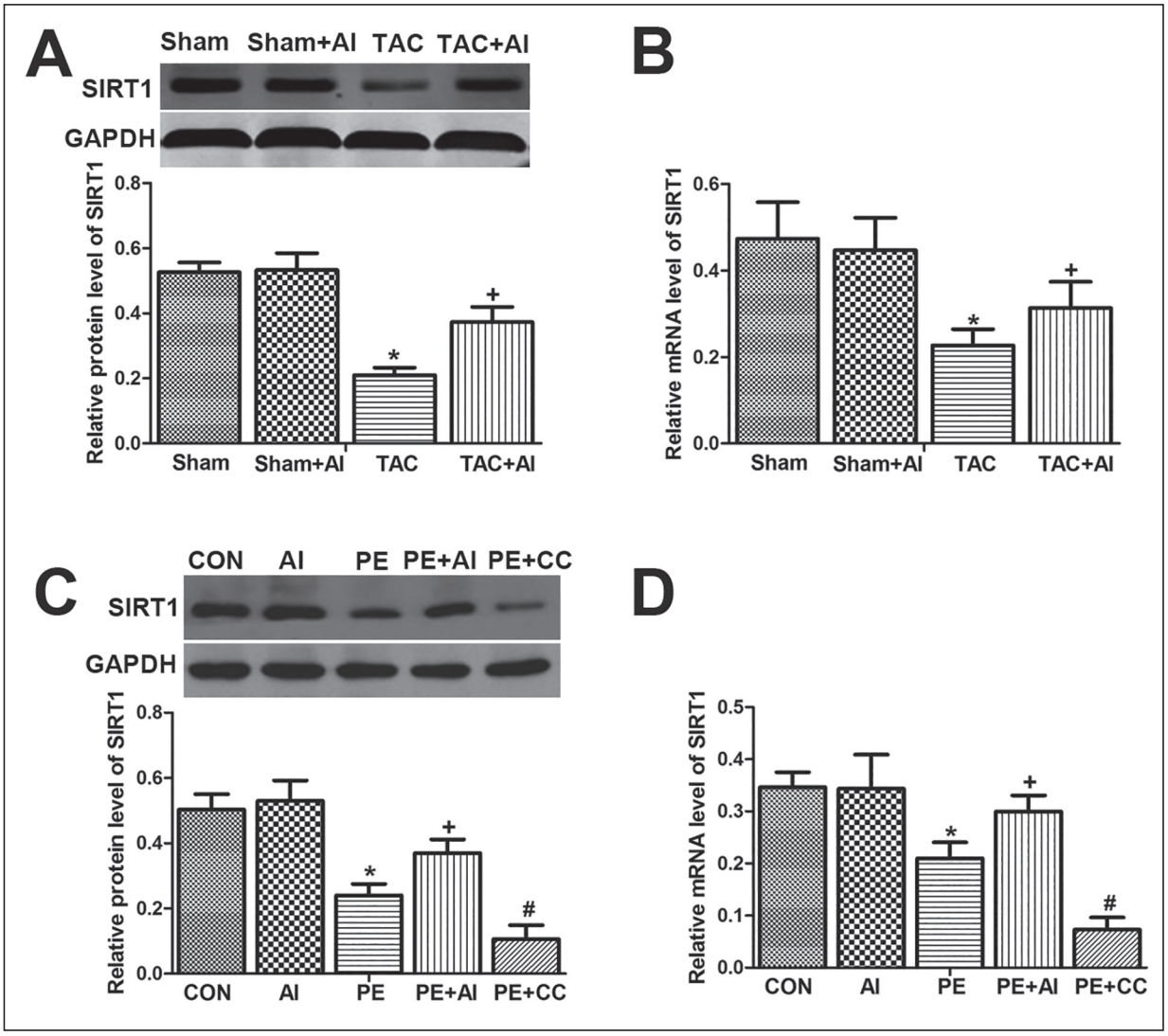

Figure 4. The changes of expression of SIRT1 in hypertrophic myocardium. ***A,*** Analysis of protein level of SIRT1 by Western blot *in vivo*. ***B,*** Analysis of mRNA level of SIRT1 by RT-PCR *in vivo*. ***C,*** Analysis of protein level of SIRT1 by Western blot *in vitro*. ***D,*** Analysis of mRNA level of SIRT1 by RT-PCR *in vitro*. *$p < 0.05$ *vs.* Sham group or CON group, + $p < 0.05$ *vs.* TAC group or PE group, $^{\#}$*vs.* PE group.

myocardial hypertrophy, the effect of PE on myocardial hypertrophy after AMPK was inhibited, was also investigated. Results showed that after AMPK activation was inhibited via CC, PE-induced myocardial hypertrophy was aggravated. The above results suggest that activation of AMPK can inhibit myocardial hypertrophy and improve cardiac function.

AMPK is a key factor of energy regulation, and the inhibitory effect of AMPK activation on myocardial hypertrophy may be related to its correction of energy metabolism. It is reported that energy conversion may be an important cause of myocardial hypertrophy. Several experimental studies[16-18] have shown that energy substrate utilization plays an important role in the pathogenesis of myocardial hypertrophy. Therefore, improving myocardial energy metabolism can inhibit myocardial hypertrophy. Results of this work showed that the fatty acid oxidation in hypertrophic heart of rats was decreased significantly, and AI inhibited the myocardial hypertrophy in rats and promoted the fatty acid oxidation. The results indicated that AI inhibits the myocardial hypertrophy, corrects myocardial fatty acid metabolism, and transforms the glucose metabolism in hypertrophic heart into the normal cardiac metabolic model, namely fatty acid oxidation.

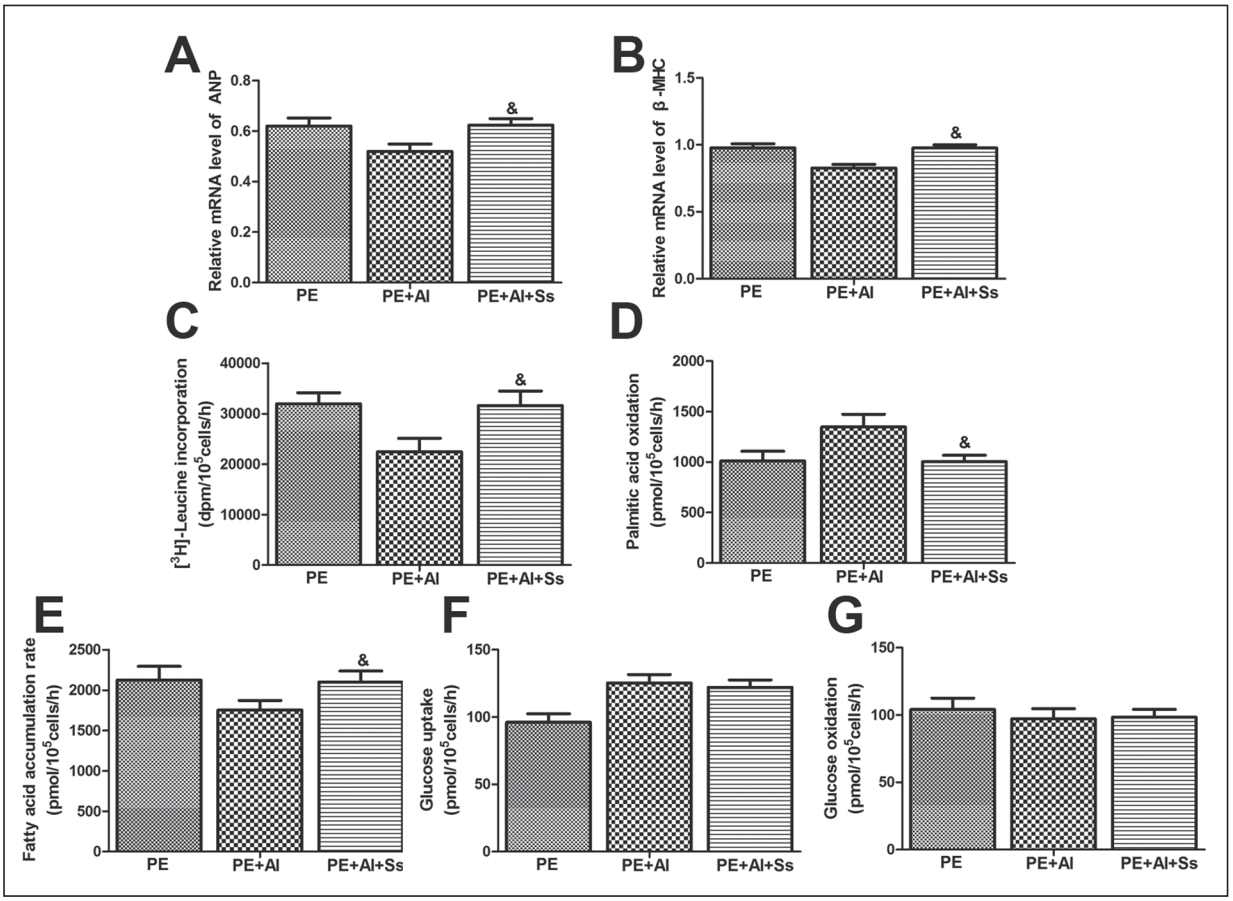

Figure 5. The role of SIRT1 in improvement of myocardial hypertrophy. ***A,*** Analysis of mRNA level of ANP *in vitro*. ***B,*** Analysis of mRNA level of β-MHC *in vitro*. ***C,*** Analysis of [^{3}H] leucine incorporation *in vitro*. ***D,*** Analysis of palmitic acid oxidation *in vitro*. ***E,*** Analysis of fatty acid deposition rate *in vitro*. ***F,*** Analysis of glucose oxidation in *in vitro*. ***G,*** Analysis of glucose uptake *in vitro*. $^{\&}p < 0.05$ *vs.* PE+AI group.

In-vitro studies showed that fatty acid oxidation is decreased in PE-induced hypertrophic myocardial cells, while the fatty acid deposition is increased. After AMPK is activated by AI, fatty acid oxidation of myocardial cells can be promoted, and the deposition of fatty acid in myocardial cells can be inhibited. These results further support the conclusion that AMPK activation can improve myocardial fatty acid metabolism and inhibit myocardial hypertrophy, which is related to the improvement in fatty acid metabolism. The relationship between changes in glucose metabolism and myocardial hypertrophy is controversial currently[19-21]. Our results showed that both glucose uptake and oxidation were increased in hypertrophic heart. The reasons may be that glucose can provide more energy than fatty acid oxidation using the same amount of oxygen, and the oxidation mode of hypertrophic myocardium is transformed from fatty acid oxidation to glucose oxidation in order to meet energy needs. The activation of AMPK via AI continues to promote the glucose uptake of myocardial tissues, but inhibits the glucose oxidation in hypertrophic myocardial tissues. It may be related to the fact that AMPK promotes fatty acid oxidation to provide more adenosine triphosphate (ATP), which inhibits glucose oxidation through the feedback path, namely the well-known Randle cycle mechanism[22]. *In-vitro* researches showed that both glucose uptake and oxidation were increased in PE-induced hypertrophic myocardial cells, and activation of AMPK via AI can promote the glucose uptake of myocardial cells without affecting the glucose oxidation in hypertrophic myocardial cells. The above result was inconsistent with that in the *in-vivo* experiments that AMPK would inhibit glucose oxidation after it was activated by AI, which may be related to the relative insufficiency of blood and oxygen supply in hy-

pertrophic heart in *in-vivo* studies. In myocardial hypertrophy, SIRT1 signaling pathway may be one of the mechanisms mediating the regulatory effect of AMPK on myocardial hypertrophy. The role of SIRT1 in regulatory effect of AMPK on myocardial hypertrophy is unclear. We found that both mRNA and protein expressions of SIRT1 were decreased in hypertrophic myocardial tissues of rats, but they were increased in AI group. These results suggest that the regulatory effect of AMPK on myocardial hypertrophy may be related to the regulation of SIRT1 activity. To confirm that the inhibitory effect of AMPK activation on myocardial hypertrophy is related to SIRT1 activation, *in-vitro* experiments were further performed. AMPK activity was inhibited by the AMPK inhibitor CC. The results showed that CC inhibited mRNA and protein expressions and transcriptional activity of SIRT1, accompanied by increased myocardial hypertrophy, suggesting that SIRT1 is a downstream regulator of AMPK, and the activation of AMPK inhibits myocardial hypertrophy through activating the SIRT1 signaling pathway. In this investigation, SIRT1 pathway was blocked by small interfering technique, and the inhibitory effect of AI on myocardial hypertrophy was observed after SIRT1 pathway was blocked, to further confirm the role of SIRT1 in inhibitory effect of AMPK activation on myocardial hypertrophy. Results showed that when SIRT1 was inhibited, the inhibitory effect of AMPK activation on myocardial hypertrophy was blocked. Moreover, the effects of AI of promoting the fatty acid oxidation in myocardial cells and inhibiting the deposition of fatty acids in myocardial cells were blocked. Fatty acid metabolism had no significant difference from glucose metabolism after SIRT1 was inhibited, indicating that SIRT1 may not participate in glucose utilization of myocardial cells. The regulatory effect of AMPK on glucose utilization of myocardial cells is probably realized via directly regulating glucose transporters. In conclusion, it is speculated that AMPK activation inhibits myocardial hypertrophy indeed through SIRT1 signaling pathway.

Conclusions

We showed that the activation of AMPK can inhibit the myocardial hypertrophy, which may be realized through regulating the myocardial energy metabolism via SIRT1 signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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