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The mechanism of BAF60c in myocardial metabolism through the PGC1 α /PPAR α /mTOR signaling pathway in rats with heart failure

Running title: BAF60c and the PGC1 α /PPAR α /mTOR axis in HF

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Abstract

BRM-associated factor (BAF) 60c promotes muscle glycolysis and improves glucose homeostasis. This study explored the mechanism of BAF60c in heart failure (HF). Fetal/adult rat models of HF were established, and the levels of cardiac contractile proteins and energy metabolism-, oxidative metabolism- and glycolysis-related factors were detected. Overexpression/siRNA BAF60c plasmids were injected into adult HF rats to estimate myocardial glucose uptake, high-energy phosphate contents, mitochondrial function, and cell proliferation and apoptosis. The overexpression/siRNA BAF60c plasmids were transfected into cardiac hypertrophic H9C2 cells to explore the *in vitro* effects. The interaction of BAF60c and PGC1 α was detected. The results suggested that adult HF rats presented increased levels of fetal proteins (ssTnI and fTnT), BAF60c and glycolysis-related factors, and reduced levels of cardiac contractile proteins, PGC1 α , PPAR α , and oxidative metabolism-related factors. BAF60c knockdown improved glucose uptake, maintained the oxidative metabolism/glycolysis balance, promoted H9C2 cell proliferation, and inhibited apoptosis. PGC1 α interacted with BAF60c. Knocking down BAF60c also activated the PGC1 α /PPAR α /mTOR pathway. Overexpression of PGC1 α decreased the damage to H9C2 cells caused by BAF60c. Altogether, BAF60c downregulation activated the PGC1 α /PPAR α /mTOR pathway, maintained the oxidative metabolism/glycolysis balance and improved mitochondrial function in rat models of HF. This study may offer novel insights into HF treatment.

Key words: Heart failure; Oxidative metabolism/glycolysis balance; Mitochondrial function; BAF60c; PGC1 α /PPAR α /mTOR pathway; Myocardial hypertrophy

Introduction

Although “energy deficiency” is considered a factor in the transition from functional compensatory hypertrophy to heart failure (HF), the details of the metabolic reconstruction and the determination of the aspects that are compensatory or inadaptable are essential for the development of new therapies (O'Rourke, Van Eyk & Foster, 2011). Based on the above evidence, we aimed to identify novel approaches for the treatment of HF from the perspective of energy metabolism.

BRM-associated factor (BAF) complexes are adenosine triphosphate (ATP)-dependent and are correlated with many aspects of heart development, specifically with cardiac transcription factors that regulate cardiac gene expression (Sun et al., 2018). BAF60c, also known as Smarcd3, is mainly expressed in skeletal muscle, brain, and heart and is considered as a critical regulator of heart development, glycolytic metabolism and glucose homeostasis (Wang et al., 2018). BAF60c is essential for function of BAF chromatin remodeling complexes in heart formation and development within pathways that regulate expansion of the heart at its poles (Lickert et al., 2004). BAF60c is crucial to coordinate gene expression programs that regulate the basic functional characteristics of cardiomyocytes (Sun et al., 2018). BAF60c can induce ectopic differentiation of mouse mesoderm into beating cardiomyocytes (Takeuchi & Bruneau, 2009).

In addition, peroxisome proliferator-activated receptors (PPARs) are mainly expressed in adipocytes, hepatocytes, muscles and endothelial cells, which exert critical roles in metabolism, especially in lipid and glucose homeostasis, so they are also called lipid and insulin sensors (Grygiel-Gorniak, 2014). Within the PPAR superfamily, PPAR α is the first cloned member, and it modulates metabolic homeostasis, induces hepatic fatty acid oxidation and regulates glucose production in the liver (Kersten, 2014). Evidence has shown that PPAR α agonists are able to attenuate HF progression and increase survival in rats (Linz et al., 2009). PPAR γ is highly expressed in white adipose tissue, in which PPAR γ regulates adipogenesis and energy balance, and PPAR γ is activated by lipids to stimulate the expression of genes implicated in lipid and glucose metabolism, thus promoting lipid accumulation (Ryan et al., 2011). PPAR γ coactivator-1 α (PGC1 α) is highly expressed in the brain, heart, liver, and kidney where there is a high energy demand, and plays pivotal roles in mitochondrial respiration, glucose transport, glycogenolysis, fatty acid oxidation, oxidative phosphorylation, and the regulation of reactive oxygen species (ROS) and superoxide contents (Corona & Duchon, 2015). Arany *et al.* found that endogenous PGC1 α has a cardioprotective function, and PGC1 α alleviates the development of HF (Arany et al., 2006). Interestingly, BAF60c could regulate hepatic fatty acid β -oxidation along with PPAR α and PGC1 α (Wang et al., 2018). Additionally, mammalian target of rapamycin (mTOR) is essential for the regulation of protein synthesis, cardiomyocyte proliferation and energy metabolism in heart

development (Zhang et al., 2014). BAF60c drives glycolytic metabolism in muscle and improves glucose homeostasis in the whole body through Deptor (an important modulator of mTOR)-mediated Akt activation (Meng et al., 2013). Based on the above evidences, we hypothesized that there may be an interaction between BAF60c and the PGC1 α /PPAR α /mTOR pathway in myocardial metabolism in rat model of HF.

Materials and methods

Ethics statement

This study was approved by the Clinical Ethical Committee of The First Affiliated Hospital of Baotou Medical College, Inner Mongolia Baotou Medical University. All experiments were conducted in accordance with the ethical guidelines for the care and use of laboratory animals (NIH Pub. No. 85–23, revised 1996) compiled by the National Institutes of health used for the study of experimental pain in conscious animals. All efforts were made to minimize the suffering of conscious animals.

Establishment of fetal/adult rat models of HF

A total of 24 fetal Sprague Dawley (SD) rats (1–3 days old) and 99 adult male SD rats (2 months old) that were provided by the experimental animal center of Chongqing Medical University (Chongqing, China) were enrolled in this study. Fetal rats were assigned to the sham group (n = 12) and HF group (n = 12). Rats were subcutaneously injected with isoproterenol (ISO) (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) at a dosage of 3 mg/kg/day (Liu et al., 2019) for 28 days for model establishment (ISO is a β -adrenergic agonist, and the hyperactivity of β -adrenergic receptor can cause heart failure. Its mechanism is closely related to oxidative stress (Zhang et al., 2005; Krennek et al., 2009). Rats in the sham group were subcutaneously injected with the same volume of saline, and the injection cycle was the same as that in the HF group. After modeling, all rats in the sham group and 12 rats in the HF group were anesthetized by an intraperitoneal injection of 2% pentobarbital sodium (0.2 mL/100 g, Shanghai Pharmaceutical Group Co., Ltd., Shanghai, China). The rats were depilated, and the skin was prepared for echocardiography. After that, the rats were euthanized by an intraperitoneal injection of pentobarbital sodium at a dosage of 800 mg/kg (Kopaladze, 2000; Zatroch et al., 2017). Then, the myocardial tissues from three rats were used for transmission electron microscopy (TEM), and myocardial tissues from another 3 rats were used for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis, and myocardial tissues from the remaining six

rats were used for hematoxylin and eosin (HE) staining to confirm the success of the model. The grouping and treatment of the adult rats were the same as that described above.

Echocardiography

After rat anesthesia was administered with [an](#) intraperitoneal injection of 2% pentobarbital sodium at 0.2 mL/100 g, echocardiography (ViVid E9, General Electric Company, Schenectady, NY, USA) and a 8-MHz wire probe were used to measure the left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS).

HE staining

The heart tissues of rats were fixed with 4% paraformaldehyde solution, dehydrated, cleared, immersed in paraffin, embedded and sliced. After HE staining (Sigma-Aldrich), the morphological changes in the heart tissues were observed under a light microscope (Olympus Optical Co., Ltd, Tokyo, Japan).

Myocardial ultrastructure observation by TEM

The heart tissues were cut into 1 mm³ sections, fixed with 2% glutaraldehyde at 4°C for 24 hours, and then fixed with 1% osmium acid for 1 hour. Next, the sections were washed, dehydrated using acetone, embedded in epoxy resin, and sliced into 90 nm sections. The ultrastructural changes were observed and photographed under TEM (Olympus, U-RFL-T).

Grouping of adult rat model of HF

The adult rats with HF were randomly allocated into five groups with 15 rats in each group. The rat models of HF were used as the HF group. On the 29th day after ISO injection, the rats in the negative control (NC) group were injected with empty BAF60c plasmid (Thermo Fisher Scientific, Waltham, MA, USA) via the caudal vein, and the other treatments were the same as those given to the HF group; the rats in the BAF60c group were injected with the BAF60c overexpression plasmid (Thermo Fisher Scientific); the rats in the si-NC group were injected with empty siRNA plasmid via the caudal vein similarly to the HF group; the rats in the si-BAF60c group were injected with the BAF60c siRNA plasmid (Thermo Fisher Scientific). All the above plasmids were obtained from Shanghai GenePharma Co., Ltd. (Shanghai, China). Each plasmid was injected at a dose of (4 mg/kg body weight) ([Hu et al., 2019](#)) and delivered with Invivoctmine 3.0 (IVF3005; Thermo Fisher Scientific).

On the 36th day after ISO injection, the rats were euthanized. The myocardial tissues of the five rats were used for tissue homogenate preparation and the detection of various indices in the homogenate. The myocardial tissues of five rats were used for histological detection, and the tissues of five other rats were used for the detection of myocardial glucose uptake.

Determination of high-energy phosphate contents

The heart was removed, and the atrial and right ventricles were separated and homogenized by centrifugation at 750 g for 10 minutes. The supernatant was centrifuged at 12,000 g for 10 minutes. The precipitate was suspended in 2 mL separation medium and centrifuged at 12,000 g for 5 minutes. The supernatant was discarded, and the precipitate was dissolved in 0.4-0.6 mL separation medium to prepare a 10 mg/mL mitochondrial suspension. The partial mitochondrial suspension was obtained and incubated on ice for 5 minutes to rupture the cell membrane. Then, the suspension was centrifuged at 15,984 g and 4°C for 20 minutes. The supernatant was absorbed and mixed with K₂CO₃. The pH of the mixture was adjusted to 6.5-7.0, and the mixture was placed on ice for 5 minutes and then centrifuged at 15,984 g and 4°C for 10 minutes. Finally, 20 µL supernatant was separated using high-pressure liquid chromatography (Waters Corp., Millford, MA, USA) to determine the contents of ATP and phosphocreatine (PCr).

Determination of the mitochondrial membrane potential (MMP)

A volume of 100 µL mitochondrial suspension was added to 1 mL reaction medium, which was allowed to react at room temperature for 5 minutes and then centrifuged at 5000 g for 5 minutes with the supernatant absorbed. Then, the concentration of rhodamine 123 outside of the mitochondria ([X]_{out}) at 486/528 nm was measured by a colorimetric assay. The volume of the mitochondrial matrix was 1 µL/mg, and the concentration of rhodamine 123 in mitochondria ([X]_{in}) was determined with the equation $(1 - 1.1 \times [X]_{out}) \times 10000/\text{protein concentration}$. The MMP (in mV) = $59 \lg ([X]_{in}/[X]_{out})$ (Baracca et al., 2003).

Determination of the oxidative respiratory activity of mitochondria

The oxidative respiratory activity of mitochondria was measured using a biological tissue oxygen consumption meter (Strathkelvin 782 system, Strathkelvin Instruments, Glasgow, Lanarkshire, Scotland, UK). Then, 0.7 mL reaction medium was added to the reaction tank and incubated for 2 minutes, and 0.1 mL mitochondrial suspension was added prior to 1-2 minutes of preheating. Next, 10 µL of reaction substrate (mixture of 1.25 mmol/L sodium malate and pyruvate) and 5 µL adenosine diphosphate (ADP) (50 mmol/L) were added successively. The oxygen consumption curve was recorded, and the oxygen consumption after the addition of ADP (state 3 respiration, ST3) and the depletion of ADP (ST4) was calculated. The oxidative respiratory activity of the mitochondria was expressed as the consumption of mitochondrial protein in nmol/min/mg, and the respiratory control rate (RCR) was expressed as the ratio of state III to state IV oxygen consumption (ST3/ST4).

Determination of mitochondrial respiratory chain oxidase activity

The activities of the nicotinamide adenine dinucleotide (NADH) enzyme, succinate oxidase (SO), and cytochrome C oxidase (CCO) (all from Sigma-Aldrich) were determined using the oxygen electrode method with a 700 μ L reaction system at 30°C (Singer, 1974).

Detection of myocardial glucose uptake

Myocardial glucose uptake was measured by 18F-fluorodeoxyglucose (FDG) myocardial perfusion imaging. Static positron emission tomography (PET) (Siemens Healthcare Diagnostic Inc., Tarrytown, NY, USA) and composite computed tomographic (CT) imaging were used. In detail, 18F-FDG was injected into the tail vein of the rats, and the rats were anesthetized 1 hour later. Then, PET was used to scan the rats and locate the 18F-FDG, and CT was used for whole-body scans. Finally, the rats were anesthetized immediately using cervical dislocation. The heart was removed by thoracotomy, and 18F-FDG γ was counted using a liquid scintillation instrument.

Wheat germ agglutinin (WGA) staining

The heart tissue sections were dewaxed and hydrated, and WGA working solution (Nanjing SenBeiJia Biological Technology Co., Ltd., Nanjing, Jiangsu, China) at 10 μ g/mL was added dropwise onto the surfaces of heart tissues, which were incubated at room temperature for 1 hour and sealed with 4',6-diamidino-2-phenylindole (DAPI).

5-Ethynyl-2'-deoxyuridine (EdU) labeling assay

The heart tissue sections were dewaxed, hydrated and then placed into boiling citrate buffer (pH = 6.0). After high-pressure repair was performed for 2 minutes, the sections were allowed to cool to room temperature, and then 100 μ L glycine (2 mg/mL) was added, after which the sections were incubated in a shaker for 5 minutes. Then, the glycine was discarded. An appropriate amount of Triton-X100 was added to 1% goat serum blocking solution. The specific procedures were carried out in strict accordance with the instructions of the EdU proliferation test kit (Guangzhou RiboBio Co., Ltd., Guangzhou, Guangdong China).

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay

A TUNEL kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) was used to detect H9C2 cell apoptosis in rats. The specific steps were performed strictly in accordance with the instructions of the kit.

Grouping of H9C2 cells

H9C2 cells (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) (HyClone Company, Logan, UT, USA) and 1% penicillin and streptomycin (Invitrogen Inc.,

Carlsbad, CA, USA) at 37°C in 5% CO₂. H9C2 cells with good growth status were taken. BAF60c siRNA plasmid, si-NC plasmid, BAF60c overexpression plasmid, BAF60c empty plasmid and PGC1α overexpression plasmid were transfected into H9C2 cells respectively according to the instructions of Lipofectamine 3000 kit (Life Technologies, Gaithersburg, MD, USA). After 24 hours, the H9C2 cells were treated with 10 μM ISO for 24 hours (Zhang et al., 2019) for subsequent experiments.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

A single cell suspension of H9C2 cells in each group was seeded at 10³-10⁴ cells/well into 96-well culture plates, which were incubated in a 5% CO₂ incubator at 37°C. Each well received 20 μL MTT solution (5 mg/mL, Sigma-Aldrich), and the cells were cultured for another 4 hours. After aspiration of the culture solution from the wells, dimethyl sulfoxide solution (150 μL/well) was added to the cells, and the culture plates were placed on a microporous plate oscillator for 10 minutes to dissolve the crystals. Three duplicated wells were used for the samples in each group. Then, the optical density (OD) value of each well at a 490 nm wavelength was detected with a microplate reader and recorded.

Detection of the H9C2 cell surface area

H9C2 cells were obtained from each group and cultured in 6-well plates at 1.5 × 10⁵ cells/well. Then, the 6-well plates were removed from the incubator, the supernatant was discarded, and the plates were washed with phosphate-buffered saline (PBS). Next, the cells were fixed with 4% paraformaldehyde for 15 minutes and washed with ddH₂O. Then, the cells were treated with ammonium oxalate-crystal violet dye (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). After 15 minutes, the dye solution was discarded, and the cells were washed with ddH₂O. The staining of cells was observed under an optical microscope (Olympus, U-RFL-T). The surface area of the H9C2 cells was measured by ImageJ 1.48 software (National Institutes of Health, Bethesda, MD, USA).

Colony formation assay

The H9C2 cells in each group in the logarithmic growth period were detached using 0.25% trypsin, dispersed into a single cell suspension, and then cultured in DMEM with 10% FBS. Next, the cell suspension was diluted in according to a concentration gradient. The cells in each group were seeded into culture dishes (50, 100 and 200 cells in each dish) and cultured in an incubator containing 5% CO₂ at 37°C. When there were visible colonies in the culture dishes, the culturing was terminated. After that, the cells were fixed with 4% paraformaldehyde for 15 minutes and then stained with Giemsa staining solution for 10-30 minutes. The staining solution was washed away slowly with running water, and the number of cell colonies was counted.

Flow cytometry

H9C2 cell apoptosis was detected in each group in strict accordance with the instructions of the annexin V-fluorescein isothiocyanate kit (Miltenyi Biotech, Bergisch Gladbach, Germany).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the heart tissue homogenate of 3 rats or H9C2 cells by TRIzol (Takara Bio Co., Ltd., Japan). The concentration and purity of the RNA were detected by an ultramicro spectrophotometer (Shanghai Puyuan Instrument Co., Ltd., Shanghai, China), and 1 µg RNA was reverse transcribed into cDNA using a reverse transcription kit (Thermo Fisher Scientific). mRNA expression was determined by the $2^{-\Delta\Delta CT}$ method with β -actin as an internal reference. All primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) ([Table I](#)). Each sample was tested three times independently.

Western blot analysis

The tissues or H9C2 cells were added to radioimmunoprecipitation assay cell lysis buffer (Jiangsu KeyGEN Biotech Corp., Ltd., Nanjing, Jiangsu, China), homogenized and centrifuged to extract the protein. The bicinchoninic acid method was used to determine the protein concentration, and 30 µg protein samples were used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then, the proteins were transferred onto membranes, which were blocked with 5% skim milk powder at room temperature and incubated with the diluted primary antibodies overnight at 4°C. Afterwards, the membranes were washed, and incubated with the secondary antibody horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G (IgG) (1:2000, ab205718) or goat anti-mouse IgG (1:10000, ab205719) at room temperature for 1 hour. The membranes were developed using enhanced chemiluminescence working solution (EMD Millipore Corporation, Billerica, MA, USA). Image Pro Plus 6.0 (Media Cybernetics, Bethesda, Maryland, USA) was used to analyze the gray value of each band in Western blot image, and β -actin was used as an internal reference. All antibodies were obtained from Abcam Inc. (Cambridge, MA, USA). The primary antibodies used for the Western blot analysis are shown in [Table II](#).

Detection of the MMP in H9C2 cells

H9C2 cells from each group were seeded into a 20 mm² special laser confocal dish and cultured for 24 hours. DMEM containing 10 µg/mL JC-1 fluorescent probes (Sigma-Aldrich) was added in the dark to the H9C2 cells, which were incubated for 40 minutes while avoiding exposure to light. After washing the dye solution with PBS, the ratio of the red fluorescence intensity to the green fluorescence intensity was analyzed using LASAF Lite2.6 software (Leica TCS SP5, Leica Inc., Germany).

Detection of the ROS content in H9C2 cells

The H9C2 cells of each group were seeded into 48-well plates, and 250 μ L DMEM containing 5 μ mol/L dihydroethidium (DHE) (Sigma-Aldrich) was added into each well and incubated at 37°C for 30 minutes. Then, 250 μ L DMEM was added to each well, which was photographed with an ArrayScanVTI high-content screening system (Shenzhen East Test Technology Co., Ltd., Shenzhen, Guangdong, China). The fluorescence intensity of DHE was measured, and the relative content of ROS was calculated.

Determination of myocardial mitochondrial superoxide content

H9C2 cells (10^5 /mL) from each group were seeded into a 20 mm² special laser confocal dish, and the non-adherent cells were washed away using DMEM. Then, the cells were added to diluted MitoSox red dye solution (YEASEN Biotechnology Co., Ltd., Shanghai, China) and incubated at 37°C for 10 minutes while avoiding exposure to light. The excess dye solution was removed using DMEM. Next, the cells were stained with 10 μ mol/L DAPI at room temperature for 5 minutes. After washing, the staining was observed under a laser confocal microscope. LASAF Lite2.6 software was applied to analyze the MiTo Sox Red fluorescence intensity/DAPI fluorescence intensity, which reflected the relative superoxide levels in the myocardial mitochondria.

Detection of [Ca²⁺]_i in H9C2 cells

H9C2 cells from each group were seeded into a 20 mm² special laser confocal dish for 24 hours. Under dark conditions, 100 μ L of high-glucose DMEM containing 5% Pluronic F-127 and 10 μ mol/L Fluo-3/AM (Sigma-Aldrich) was added to each dish, which was incubated for 40 minutes. The excess dye solution was removed with PBS, and the cells were observed under a laser confocal microscope. The relative [Ca²⁺]_i in the H9C2 cells was directly proportional to the average fluorescence intensity. The experimental results were analyzed by ImageJ software.

Coimmunoprecipitation assay

The coimmunoprecipitation assay was performed according to the instructions of the Pierce Classic IP kit (Beyotime).

Statistical analysis

Statistical analysis was conducted using SPSS 21.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to verify that the data were normally distributed. The measurement data are presented as the mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) or two-way ANOVA was applied to analyze the comparisons among multiple groups, followed by Tukey's multiple comparisons test or Sidak's multiple comparisons test, respectively. The *p* value was determined by a two-tailed test, and *p* < 0.05 indicated a significant difference.

Results

In adult rat model of HF, the expression of contractile proteins were decreased

To explore the phenotypic changes of fetal/adult HF rats, we induced fetal/adult HF rats (HF group) with ISO, and rats injected with saline with no HF were used as control (sham group). At first, the cardiac function of rats was measured. The results showed that compared with the values in the sham group, the LVEF and LVFS in the HF group were reduced, and the LVEDD and LVESD were increased (all $p < 0.05$) (Figure 1A). In addition, in the rats in the HF group, cardiomyocytes were disorderly arranged, which was accompanied by myocardial fibrosis, cardiac hypertrophy and mitochondrial swelling, while rats in the sham group showed normal nuclear morphology, orderly arrangement of cardiomyocytes and no fibrosis of myocardium (Figure 1B-C). The results showed that the rat models of fetal/adult HF were successfully established.

In addition, RT-qPCR was used to detect the changes of contractile proteins in adult HF rats. The results revealed that compared with sham operated rats, adult HF rats exhibited the obviously increased mRNA expressions of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in heart tissues, and the dramatically altered expression of cardiac contractile proteins [α -myosin heavy chain (α -MHC) and cardiac troponin I (cTnI) were reduced, while β -myosin heavy chain (β -MHC), slow skeletal troponin I (ssTnI), and fast skeletal troponin T (fTnT) were increased] (all $p < 0.05$).

In the same way, RT-qPCR was used to detect the changes of contractile proteins in the heart tissues of fetal HF rats. We found that relative to those in the sham-operated rats, the mRNA expressions of ANP and BNP were significantly decreased, and the α -MHC and cTnI were remarkably increased, and β -MHC, ssTnI, fTnT were notably decreased (all $p < 0.05$) (Figure 1D) in fetal HF rats. The above results indicated that the compensatory mechanism of adult HF rats was opposite to that of fetal HF rats.

In adult HF rats, BAF60c expression was increased, while PGC1 α and PPAR α were decreased

To further study the differences in energy metabolism in fetal/adult HF rats, we detected the expression changes of energy metabolism regulatory factors. The results revealed that compared to those in the sham group, the levels of BAF60c were increased, while levels of PPAR α and PGC1 α were noticeably decreased in adult HF rats (all $p < 0.05$) (Figure 2A/B). Fetal HF rats showed an opposite trend, as manifested as the obvious decrease of BAF60c mRNA and protein levels, and the increase of PPAR α and PGC1 α mRNA and protein levels (all $p < 0.05$) (Figure 2A/B).

In addition, we also found that there were differences in oxidative metabolism/glycolysis in fetal/adult HF rats. Compared with that in the sham group, the mRNA expression of oxidative metabolism-related factors, such as mitochondrial creatine kinase (mCK), medium chain acyl-CoA

dehydrogenase (MCAD), acetyl coenzyme A carboxylase (ACC)1, and carnitine palmitoyl transfer 1 (CPT-1), was significantly decreased, while uncoupling protein-3 (UCP3) and pyruvate dehydrogenase kinase isozyme 4 (PDK4) expression was greatly increased in adult HF rats. The mRNA expression of glycolysis-related factors hexosaminidase 2 (Hexo2), phosphofructokinase muscle specific isoform (PFK-M) and aldolase was significantly increased in adult HF rats (all $p < 0.05$) (Figure 2C/D). However, the mRNA expression of mCK, MCAD, ACC1 and CPT-1 was notably elevated, and the mRNA expression of UCP3, PDK4, Hexo2, PFK-M and aldolase was significantly decreased in fetal HF rats (all $p < 0.05$) (Figure 2C/D). These alterations indicated that the oxidative metabolism/glycolysis compensation mechanism in fetal HF rats was opposite to that in adult HF rats.

Interference with BAF60c improved high-energy phosphate metabolism and mitochondrial function in adult HF rats

To further explore the effect of BAF60c on HF rats, we injected BAF60c overexpression plasmids/siRNA plasmids (BAF60c/si-BAF60c) into adult HF rats to upregulate/downregulate BAF60c expression with NC/si-NC as controls, and the transfection was verified by RT-qPCR (Figure 3A). In response to the decrease in expression of BAF60c in HF rats, the ratio and amount of myocardial glucose uptake, cardiac ATP concentration, PCr/ATP, and levels of MMP, ST3, PCr, mitochondrial respiratory chain oxidase activity, SO, CCO, and NADH oxidase were significantly increased, while the level of ST4 was decreased (all $p < 0.05$) (Figure 3B-G). In addition, after increasing the expression of BAF60c in HF rats, the ratio and amount of myocardial glucose uptake, cardiac ATP concentration, PCr/ATP, and levels of MMP, ST3, PCr, mitochondrial respiratory chain oxidase activity, SO, CCO, and NADH oxidase were obviously decreased, while the level of ST4 was increased (all $p < 0.05$) (Figure 3B-G).

Additionally, we also found through the results of the EdU, WGA and TUNEL assays that in response to a decrease in expression of BAF60c in HF rats, the number of cardiomyocytes was increased, and the number of TUNEL-positive cells was significantly decreased (all $p < 0.05$); similarly, BAF60c overexpression in HF rats remarkably reduced the number of cardiomyocytes and increased the number of TUNEL-positive cells (Figure 4A/B/C).

BAF60c knock-down maintained the balance in oxidative metabolism and glycolysis in H9C2 cells

From the above results *in vivo*, interference with BAF60c expression may have a certain effect on HF rats. To further confirm the effect of BAF60c on HF, we conducted *in vitro* experiments. The H9C2 cells were induced with ISO, and transfected with BAF60c overexpression/siRNA plasmids (BAF60c/si-BAF60c) (Figure 5A). The results of crystal violet staining and RT-qPCR revealed that

the surface area of H9C2 cells was obviously reduced, the mRNA expression of the oxidative metabolism-related factors mCK, MCAD, ACC1, and CPT-1 was enhanced, while UCP3 and PDK4 mRNA expressions were reduced, and the expressions of the glycolysis-related factors Hexo2, PFK-M, aldolase and GAPDH were markedly decreased after the decrease in the expression of BAF60c. However, in cells with BAF60c overexpression, the surface area was significantly increased, the mRNA expression of mCK, MCAD, ACC1, and CPT-1 was notably decreased, the mRNA expression of UCP3 and PDK4 was increased, and the mRNA expression of glycolysis-related factors Hexo2, PFK-M, aldolase and GAPDH was dramatically increased (all $p < 0.05$) (Figure 5B-D).

BAF60c knock-down improved high-energy phosphate metabolism and mitochondrial function in H9C2 cells, and promoted cell proliferation and inhibited apoptosis

Compared with those in the si-NC group, H9C2 cells in the si-BAF60c group exhibited the remarkably increased proliferation ability, decreased apoptosis rate and the expression of pro-apoptotic proteins (Caspase-3 and Bax), and elevated expression of anti-apoptotic protein Bcl-2, while H9C2 cells in the BAF60c group the notably increased apoptosis rate and the expression of Caspase-3 and Bax, along with the obviously decreased Bcl-2 expression (all $p < 0.05$) (Figure 6A-D).

High-pressure liquid chromatography was used to study the metabolism of high-energy phosphate in H9C2 cells of each group. The ATP concentration, PCr/ATP and MMP level in H9C2 cells in the si-BAF60c group were substantially increased, while H9C2 cells in the BAF60c group showed the opposite trend, as confirmed by the obviously reduced ATP concentration, PCr/ATP and MMP level (all $p < 0.05$) (Figure 6E-F). In addition, the function of mitochondria in H9C2 cells was studied. The results showed that when the expression of BAF60c was decreased, the ratio of the red fluorescence intensity to green fluorescence intensity, which reflected the MMP, was increased, while the contents of superoxide and intracellular ROS and the fluorescence intensity of $[Ca^{2+}]_i$ were significantly decreased (all $p < 0.05$). On the contrary, after overexpression of BAF60c in H9C2 cells, the ratio of the red fluorescence intensity to green fluorescence intensity was reduced, and the contents of superoxide and intracellular ROS and the fluorescence intensity of $[Ca^{2+}]_i$ were increased (all $p < 0.05$) (Figure 7A-D).

BAF60c knock-down activated the PGC1 α /PPAR α /mTOR signaling pathway

The above *in vivo* and *in vitro* experiments confirmed that inhibition of BAF60c expression had protective effects on HF. However, the downstream mechanism of BAF60c was still unclear. A previous study has shown that overexpression of PGC1 α can partially rescue chronic HF in cardiomyocyte-specific RMP-deficient mice (Zhang et al. 2019a). Therefore, we speculated that

BAF60c and PGC1 α may interact to influence the occurrence and development of HF. To confirm this speculation, we carried out a coimmunoprecipitation assay, and we found that BAF60c could bind to PGC1 α . In addition, RT-qPCR showed that in H9C2 cells in the si-BAF60c group, the protein levels of PGC1 α and PPAR α were increased, and the phosphorylation levels of proteins related to the mTOR pathway were also noticeably increased; while the protein levels of PGC1 α and PPAR α and the phosphorylation levels of proteins related to the mTOR pathway were remarkably reduced in H9C2 cells in the BAF60c group (Figure 8A-C). The above results indicated that BAF60c knock-down activated the PGC1 α /PPAR α /mTOR pathway.

Overexpression of PGC1 α reduced the damage to H9C2 cells caused by BAF60c

To confirm that BAF60c plays a role through the PGC1 α /PPAR α /mTOR signaling pathway, we carried out functional rescue experiments. We found that compared with those in H9C2 cells in the BAF60c group, the surface area of H9C2 cells was notably reduced, the proliferation of H9C2 cells was promoted and the apoptosis was inhibited in H9C2 cells in the BAF60c + PGC1 α group, along with the increased expression of the energy metabolism-related factors mCK, MCAD, ACC1, and CPT-1, decreased expression of UCP3 and PDK4, and significantly reduced expression of glycolysis-related factors (Hexo2, PFK-M and GAPDH) (Figure 9A-E). Meanwhile, H9C2 cells in the BAF60c + PGC1 α group also showed elevated concentrations of ATP, PCr/ATP and MMP (Figure 9F), as well as decreased contents of superoxide and intracellular ROS and fluorescence intensity of [Ca²⁺]_i (all $p < 0.05$) (Figure 9G-H).

Discussion

Before implementing the present study, we hypothesized that BAF60c and the PGC1 α /PPAR α /mTOR pathway may interact to affect myocardial metabolism in rat model of HF. As expected, our results concluded that BAF60c knockdown maintained the oxidative metabolism/glycolysis balance and improved mitochondrial function in rat model of HF by activating the PGC1 α /PPAR α /mTOR pathway.

First, we found that in adult rat model of HF, ANP and BNP in cardiac tissues increased, and cardiac contractile proteins (α -MHC and cTnI) decreased, while β -MHC, ssTnI and fTnT increased. HF is associated with elevated plasma ANP and BNP levels, accompanied by progressive contractile dysfunction (Tripathi et al., 2016). Myocardial infarction-induced rat model of HF showed decreased α -MHC and increased β -MHC contents and decreased myofibrillar Ca²⁺-stimulated ATP activity and cTnI phosphorylation (Machackova et al., 2011). The ssTnI protein subtype was overexpressed in the hearts of adult mice and increased the sensitivity of

myofilaments to Ca^{2+} (Ford & Chandra, 2012). Additionally, in adult rat model of HF, BAF60c was increased, while PGC1 α and PPAR α expressions were decreased; the expressions of the oxidative metabolism-related factors were decreased, while the expressions of the glycolysis-related factors were increased. Mitochondrial and cardiac energy metabolism disorders are contributors to HF development (Dolinsky et al., 2016). BAF60c expression was adequate to activate the molecular, metabolic and contractile alterations of fast twitch glycolysis and stimulate a switch from oxidative to glycolytic metabolism (Meng et al., 2013). Baf60c initiated ectopic cardiac gene expression (Takeuchi & Bruneau, 2009). In PPAR α knockout mice, palmitate oxidation was decreased and was accompanied by increased rates of glucose oxidation and glycolysis (Dolinsky et al., 2016). PGC1 α regulated the fundamental transcriptional cycle that modulated cellular respiration and mitochondrial function and allowed homeostatic control of ATP and cell survival (Rohas et al., 2007). The PPAR- α /sirtuin 1/PGC1 α pathway was significantly inhibited in patients with atrial fibrillation (Liu et al., 2016). To sum up, elevated BAF60c expression was accompanied with imbalance of oxidative metabolism and glycolysis in rat model of HF.

Furthermore, BAF60c knockdown increased myocardial glucose uptake, mitochondrial function, and expression of oxidative metabolism-related factors, while it decreased the expression of glycolysis-related factors and the contents of ROS. The increase in glycolytic metabolism is a kind of compensatory response to the increase in blood glucose, while deficient glycolysis metabolism possibly speeds up diabetic progression (Wang et al., 2018). Reduced cardiac ATP levels occur after mitochondrial damage and MMP loss, accounting for HF (Pointon et al., 2010; Chen et al., 2012). The PCr/ATP, reflecting the mitochondrial energy reserve status, was reduced in animal models of HF (Chen et al., 2012). Disruption of MMP induced the release of apoptotic proteins, and the improvement of mitochondrial function is synonymous with a reduction in ROS production (Guo et al., 2012). Inhibition of the oxidative metabolism-related factors is effective in reducing HF morbidity in animal studies (O'Rourke et al., 2011). In addition, BAF60c knockdown increased H9C2 cell proliferation and suppressed apoptosis. Baf60c siRNA prevented the developmental opening of chromatin surrounding the Nkx2.5 early cardiac enhancer and cardiomyocyte differentiation, and repressed cardiac markers (Nkx2-5, bMHC, MLC2v, MLC2a, cTnT, and ANF) (Cai et al., 2013). BAF60c is necessary for the regulation of energy metabolism (Wang et al., 2018). Adenoviral-mediated BAF60a stimulated fatty acid beta-oxidation in cultured hepatocytes and ameliorated hepatic steatosis (Li et al., 2008). Overall, BAF60c downregulation maintained the balance of oxidative metabolism and glycolysis and alleviated HF.

Moreover, our results revealed that BAF60c knockdown activated the PGC1 α /PPAR α /mTOR signaling pathway. BAF60c is necessary for the regulation of energy metabolism, and similarly,

BAF60c could regulate hepatic fatty acid β -oxidation along with PPAR α and PGC1 α (Wang et al., 2018). PGC1 α activation increased the expression of mitochondrial respiratory chain proteins (Zheng et al., 2010), and adenoviral-mediated PGC1 α overexpression induced the levels of MCAD, ATP-5b and PDK4 in cardiomyocytes and inhibited cleaved caspase3 (Chen et al., 2012). The activation of fatty acid oxidase induced by PPAR agonists was accompanied by significant upregulation of ACC and CPT-1, and PPAR α could alter glucose utilization by inducing PDK1 and PDK4 expression (Kersten, 2014). The energy metabolism and expression of CPT-1, which transfers fatty acids into mitochondria for further oxidation, was decreased, and cardiomyocyte death and cardiac fibrosis were induced after the deletion of mTOR (Zhang et al., 2014). It is well known that mTOR is a master regulator of autophagy (Jia et al. 2019). In addition, PGC1 α overexpression reduced the damage to cardiomyocytes caused by BAF60c, increased the expression of energy metabolism-related factors, reduced the expression of glycolysis-related factors. PGC1 α mediates the recruitment of BAF60a to PPAR α -binding sites, leading to transcriptional activation of peroxisomal and mitochondrial fat-oxidation genes (Li et al., 2008).

In conclusion, our study provides evidence that BAF60c downregulation maintained the oxidative metabolism/glycolysis balance and improved mitochondrial function in rat model of HF by activating the PGC1 α /PPAR α /mTOR pathway. Given that the secondary sexual characteristics of fetal rats are not obvious, and it is difficult to distinguish male and female rats in appearance, therefore the sex of fetal mice was not distinguished in this study. Although in general, it is not necessary to pay much attention to the sex of fetal rats, we found in the first result that the compensatory mechanism of male adult HF rats was contrary to that of fetal rats, which may be related to the lack of confirmation of fetal rat sex. We will discuss this point in our future research. More research is urgently needed to further validate our results and develop clinical treatment approaches for HF. Due to the limitation of our experimental conditions and fund, we can't use the new method to carry out new tests at present. Moreover, as has been pointed out in a previous study, the mTOR signaling plays an essential role in cardiomyocyte apoptosis via regulating autophagy (Hu et al. 2019a). In the future research, we will focus on relationships among low expression of BAF60c, autophagy and cell apoptosis. We will also carry out experiments to check the extent of loss of dye following mitochondrial depolarization with FCCP, focus on the specific binding region sequence of Baf60c and PGC1 α and conduct in-depth exploration.

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Availability of data and materials

All the data generated or analyzed during this study are included in this published article.

Author contributions

Qiang Chen is the guarantor of the integrity of the entire study and contributed to the study concept. Lizhu Chen contributed to the study design and experiments. Jianguo Jian and Junping Li were responsible for the acquisition and analysis of data. Xiaomiao Zhang and Qiang Chen contributed to the manuscript preparation and manuscript editing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Clinical Ethical Committee of The First Affiliated Hospital of Baotou Medical College, Inner Mongolia Baotou Medical University. All experiments were conducted in accordance with the ethical guidelines for the care and use of laboratory animals (NIH Pub. No. 85–23, revised 1996) compiled by the National Institutes of health used for the study of experimental pain in conscious animals. All efforts were made to minimize the suffering of conscious animals.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that there are no conflicts of interest in regard to this study.

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Figure 1 In adult HF rats, the expression of contractile proteins was decreased. The fetal/adult rats were induced HF with ISO (HF group), and rats injected with saline with no HF were used as control (sham group). A. The cardiac function-related indices (LVEF, LVFS, LVEDD, and LVESD) of fetal/adult rats were measured by echocardiography ($n = 12$); B. HE staining was used to detect the pathological morphology of the fetal/adult rat model of HF ($\times 400$) ($n = 6$); C. The morphology of myocardial mitochondria in cardiac tissues of fetal/adult rats was observed by TEM ($\times 12500$) ($n = 3$); D. The mRNA expression of ANP and BNP and cardiac contractile proteins α -MHC, cTnI, β -MHC, ssTnI and fTnT ($n = 3$) in cardiac tissues of fetal/adult rats were detected by RT-qPCR. $**p < 0.05$ vs the sham group. Data in panels A and D were analyzed with two-way ANOVA followed by Tukey's multiple comparisons test.

Figure 2 BAF60c expression was increased, while PGC1 α and PPAR α expressions were decreased in adult HF rats. The fetal/adult rats were induced HF with ISO (HF group), and rats injected with saline with no HF were used as control (sham group). A-B. Relative mRNA (A) and protein (B) levels of BAF60c, PGC1 α and PPAR α in cardiac tissues of fetal/adult rats detected by RT-qPCR and western blot analysis ($n = 5$); C. RT-qPCR was used to detect the mRNA expression of oxidative metabolism-related factors (mCK, MCAD, ACC1, CPT-1, UCP3 and PDK4) in cardiac tissues of fetal/adult rats; D. RT-qPCR was used to detect the mRNA expression of Hexo2, PFK-M and aldolase in cardiac tissues of fetal/adult rats. $**p < 0.05$ vs the sham group. Replicates = 3. Data were analyzed with two-way ANOVA followed by Tukey's multiple comparisons test.

Figure 3 BAF60c knock-down improved high-energy phosphate metabolism and mitochondrial function in adult HF rats. The BAF60c overexpression plasmids/siRNA plasmids (BAF60c/si-BAF60c) were injected into adult HF rats to upregulate/downregulate BAF60c expression with NC/si-NC as controls. A. Relative mRNA expression of BAF60c in cardiac tissues of adult HF rats detected by RT-qPCR ($n = 5$); B. Myocardial glucose uptake in adult HF rats was measured by ^{18}F -FDG myocardial perfusion imaging ($n = 5$); C. ^{18}F -FDG γ in cardiac tissues of adult HF rats in each group was counted by liquid scintillation apparatus ($n = 5$); D. The content of high-energy phosphate in cardiac tissues of adult HF rats in each group was determined by high performance liquid chromatography ($n = 6$); E. Relative MMP level in cardiac tissues of adult HF rats in each group; F. The levels of oxidative respiration-related indexes (ST3, PCr, ST4) in cardiac tissues of adult HF rats in each group were measured by tissue oxygen consumption meter ($n = 5$); G. Relative levels of SO, CCO and NADH oxidase in mitochondrial respiratory chain in cardiac tissues of adult HF rats in each group ($n = 5$). $**p < 0.05$ for pairwise comparison. Data in panels

A/C/D/E/F/G were analyzed with one-way ANOVA followed by Tukey's multiple comparisons test.

Figure 4 BAF60c depletion improved high-energy phosphate metabolism and mitochondrial function in adult HF rats. The BAF60c overexpression plasmids/siRNA plasmids (BAF60c/si-BAF60c) were injected into adult HF rats to upregulate/downregulate BAF60c expression with NC/si-NC as controls. A. The number of EdU-positive cells in cardiac tissues of adult HF rats in each group was detected by EdU labeling assay ($\times 200$) ($n = 5$); B. Cell proliferation in cardiac tissues of adult HF rats in each group was observed by WGA staining ($n = 5$); C. TUNEL-positive cell rate in cardiac tissues of adult HF rats in each group was detected by TUNEL assay ($n = 5$). $**p < 0.05$ for pairwise comparison. Data in panels A/C were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Figure 5 BAF60c knock-down maintained the balance of oxidative metabolism and glycolysis in H9C2 cells. H2C2 cells were induced with ISO and transfected with si-BAF60c/BAF60c plasmids to downregulate/upregulate BAF60c H2C2 cells. A. Relative mRNA expression of BAF60c in H9C2 cells detected by RT-qPCR; B. The surface area of H9C2 cells was measured by crystal violet staining ($\times 400$); C. RT-qPCR was used to detect the mRNA expression of oxidative metabolism-related factors (mCK, MCAD, ACC1, CPT-1, UCP3 and PDK4) in H9C2 cells; D. RT-qPCR was used to detect the mRNA expression of Hexo2, PFK-M, aldolase and GAPDH in H9C2 cells. $**p < 0.05$ for pairwise comparison. Data in panels A and B were analyzed with one-way ANOVA, and data in panels C and D were analyzed by two-way ANOVA, both were followed by Tukey's multiple comparisons test.

Figure 6 BAF60c knock-down improved high-energy phosphate metabolism and mitochondrial function in H9C2 cells, promoted proliferation and inhibited apoptosis. H2C2 cells were induced with ISO and transfected with si-BAF60c/BAF60c plasmids to downregulate/upregulate BAF60c expression. A. MTT assay was used to detect the viability of H9C2 cells in each group; B. Colony formation assay was used to detect the ability of H9C2 cell colony formation in each group; C. Flow cytometry was used to detect the apoptosis of H9C2 cells in each group; D. The protein levels of apoptosis-related proteins in H9C2 cells were detected by western blot analysis; E. The content of high-energy phosphate in H9C2 cells was determined by high performance liquid chromatography; F. Relative MMP level in H9C2 cells in each group. $**p < 0.05$ for pairwise comparison. Replicates = 3. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Figure 7 BAF60c knock-down improved high-energy phosphate metabolism and mitochondrial function in H9C2 cells, promoted proliferation and inhibited apoptosis. H2C2 cells were induced

with ISO and transfected with si-BAF60c/BAF60c plasmids to downregulate/upregulate BAF60c expression. A. JC-1 fluorescence probe was used to detect MMP ($\times 400$) of H9C2 cells in each group. The ratio of red fluorescence intensity to green fluorescence intensity indicated the change of MMP; B. Mito Sox Red was used to detect the level of myocardial mitochondrial superoxide in H9C2 cells in each group; C. ROS content in H9C2 cells in each group was detected by DHE fluorescence probe; D. $[Ca^{2+}]_i$ in H9C2 cells was detected by Fluo-3/AM fluorescence probe ($\times 400$). $**p < 0.05$ for pairwise comparison. Replicates = 3. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Figure 8 BAF60c knock-down activated the PGC1 α /PPAR α /mTOR signaling pathway. A. The binding between BAF60c and PGC1 α was detected by coimmunoprecipitation assay; B. Relative protein levels of PGC1 α and PPAR α in H9C2 cells in each group were detected by western blot analysis; C. Protein levels of mTOR pathway-related proteins (p-mTOR, mTOR, p-p70S6K, p70S6K, p-4E-BP1, and 4E-BP1) and their phosphorylation levels in H9C2 cells in each group were detected by western blot analysis. $**p < 0.05$ for pairwise comparison. Replicates = 3. Data in panels B and C were analyzed with one-way ANOVA followed by Tukey's multiple comparisons test.

Figure 9 Overexpression of PGC1 α reduced the damage of BAF60c to H9C2 cells. H9C2 cells with BAF60c overexpression were transfected with PGC1 α overexpression plasmid. A. Crystal violet staining was used to detect the surface area of H9C2 cells in two groups ($\times 400$); B. Colony formation assay was used to detect the colony formation ability of H9C2 cells in two groups; C. Flow cytometry was used to detect the apoptosis of H9C2 cells in two groups; D. RT-qPCR was used to detect the expression of indices related to oxidative metabolism and glycolysis of H9C2 cells in two groups; E. RT-qPCR was used to detect the mRNA expression of Hexo2, PFK-M and GAPDH in two groups; F. Relative ATP and MMP levels in H9C2 cells in two groups; G. ROS content in H9C2 cells was detected by DHE fluorescence probe ($\times 400$); H. $[Ca^{2+}]_i$ in H9C2 cells in two groups was detected by Fluo-3/AM fluorescence probe ($\times 400$). $**p < 0.05$ vs the BAF60c group. Replicates = 3. Data in panels A, C, F, G, and H were analyzed with independent t test, and data in panels D and E were analyzed using two-way ANOVA followed by Sidak's multiple comparisons test.

Figure 1 In adult HF rats, the expression of contractile proteins was decreased. The fetal/adult rats were induced HF with ISO (HF group), and rats injected with saline with no HF were used as control (sham group). A. The cardiac function-related indices (LVEF, LVFS, LVEDD, and LVESD) of fetal/adult rats were measured by echocardiography (n = 12); B. HE staining was used to detect the pathological morphology of the fetal/adult rat model of HF (× 400) (n = 6); C. The morphology of myocardial mitochondria in cardiac tissues of fetal/adult rats was observed by TEM (× 12500) (n = 3); D. The mRNA expression of ANP and BNP and cardiac contractile proteins α-MHC, cTnI, β-MHC, ssTnI and fTnT (n = 3) in cardiac tissues of fetal/adult rats were detected by RT-qPCR. **p < 0.05 vs the sham group. Data in panels A and D were analyzed with two-way ANOVA followed by Tukey's multiple comparisons test.

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Figure 2 BAF60c expression was increased, while PGC1 α and PPAR α expressions were decreased in adult HF rats. The fetal/adult rats were induced HF with ISO (HF group), and rats injected with saline with no HF were used as control (sham group). A-B. Relative mRNA (A) and protein (B) levels of BAF60c, PGC1 α and PPAR α in cardiac tissues of fetal/adult rats detected by RT-qPCR and western blot analysis (n = 5); C. RT-qPCR was used to detect the mRNA expression of oxidative metabolism-related factors (mCK, MCAD, ACC1, CPT-1, UCP3 and PDK4) in cardiac tissues of fetal/adult rats; D. RT-qPCR was used to detect the mRNA expression of Hexo2, PFK-M and aldolase in cardiac tissues of fetal/adult rats. **p < 0.05 vs the sham group. Replicates = 3. Data were analyzed with two-way ANOVA followed by Tukey's multiple comparisons test.

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Figure 3 BAF60c knock-down improved high-energy phosphate metabolism and mitochondrial function in adult HF rats. The BAF60c overexpression plasmids/siRNA plasmids (BAF60c/si-BAF60c) were injected into adult HF rats to upregulate/downregulate BAF60c expression with NC/si-NC as controls. A. Relative mRNA expression of BAF60c in cardiac tissues of adult HF rats detected by RT-qPCR (n = 5); B. Myocardial glucose uptake in adult HF rats was measured by 18F-FDG myocardial perfusion imaging (n = 5); C. 18F-FDGγ in cardiac tissues of adult HF rats in each group was counted by liquid scintillation apparatus (n = 5); D. The content of high-energy phosphate in cardiac tissues of adult HF rats in each group was determined by high performance liquid chromatography (n = 6); E. Relative MMP level in cardiac tissues of adult HF rats in each group; F. The levels of oxidative respiration-related indexes (ST₃, PCr, ST₄) in cardiac tissues of adult HF rats in each group were measured by tissue oxygen consumption meter (n = 5); G. Relative levels of SO, CCO and NADH oxidase in mitochondrial respiratory chain in cardiac tissues of adult HF rats in each group (n = 5). **p < 0.05 for pairwise comparison. Data in panels A/C/D/E/F/G were analyzed with one-way ANOVA followed by Tukey's multiple comparisons test.

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Figure 4 BAF60c depletion improved high-energy phosphate metabolism and mitochondrial function in adult HF rats. The BAF60c overexpression plasmids/siRNA plasmids (BAF60c/si-BAF60c) were injected into adult HF rats to upregulate/downregulate BAF60c expression with NC/si-NC as controls. A. The number of EdU-positive cells in cardiac tissues of adult HF rats in each group was detected by EdU labeling assay ($\times 200$) (n = 5); B. Cell proliferation in cardiac tissues of adult HF rats in each group was observed by WGA staining (n = 5); C. TUNEL-positive cell rate in cardiac tissues of adult HF rats in each group was detected by TUNEL assay (n = 5). **p < 0.05 for pairwise comparison. Data in panels A/C were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

170x96mm (300 x 300 DPI)

Figure 5 BAF60c knock-down maintained the balance of oxidative metabolism and glycolysis in H9C2 cells. H2C2 cells were induced with ISO and transfected with si-BAF60c/BAF60c plasmids to downregulate/upregulate BAF60c H2C2 cells. A. Relative mRNA expression of BAF60c in H9C2 cells detected by RT-qPCR; B. The surface area of H9C2 cells was measured by crystal violet staining ($\times 400$); C. RT-qPCR was used to detect the mRNA expression of oxidative metabolism-related factors (mCK, MCAD, ACC1, CPT-1, UCP3 and PDK4) in H9C2 cells; D. RT-qPCR was used to detect the mRNA expression of Hexo2, PFK-M, aldolase and GAPDH in H9C2 cells. $**p < 0.05$ for pairwise comparison. Data in panels A and B were analyzed with one-way ANOVA, and data in panels C and D were analyzed by two-way ANOVA, both were followed by Tukey's multiple comparisons test.

170x96mm (300 x 300 DPI)

Figure 6 BAF60c knock-down improved high-energy phosphate metabolism and mitochondrial function in H9C2 cells, promoted proliferation and inhibited apoptosis. H2C2 cells were induced with ISO and transfected with si-BAF60c/BAF60c plasmids to downregulate/upregulate BAF60c expression. A. MTT assay was used to detect the viability of H9C2 cells in each group; B. Colony formation assay was used to detect the ability of H9C2 cell colony formation in each group; C. Flow cytometry was used to detect the apoptosis of H9C2 cells in each group; D. The protein levels of apoptosis-related proteins in H9C2 cells were detected by western blot analysis; E. The content of high-energy phosphate in H9C2 cells was determined by high performance liquid chromatography; F. Relative MMP level in H9C2 cells in each group. **p < 0.05 for pairwise comparison. Replicates = 3. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

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Figure 7 BAF60c knock-down improved high-energy phosphate metabolism and mitochondrial function in H9C2 cells, promoted proliferation and inhibited apoptosis. H9C2 cells were induced with ISO and transfected with si-BAF60c/BAF60c plasmids to downregulate/upregulate BAF60c expression. A. JC-1 fluorescence probe was used to detect MMP ($\times 400$) of H9C2 cells in each group. The ratio of red fluorescence intensity to green fluorescence intensity indicated the change of MMP; B. Mito Sox Red was used to detect the level of myocardial mitochondrial superoxide in H9C2 cells in each group; C. ROS content in H9C2 cells in each group was detected by DHE fluorescence probe; D. [Ca²⁺]_i in H9C2 cells was detected by Fluo-3/AM fluorescence probe ($\times 400$). ** $p < 0.05$ for pairwise comparison. Replicates = 3. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

170x72mm (300 x 300 DPI)

Figure 8 BAF60c knock-down activated the PGC1α/PPARα/mTOR signaling pathway. A. The binding between BAF60c and PGC1α was detected by coimmunoprecipitation assay; B. Relative protein levels of PGC1α and PPARα in H9C2 cells in each group were detected by western blot analysis; C. Protein levels of mTOR pathway-related proteins (p-mTOR, mTOR, p-p70S6K, p70S6K, p-4E-BP1, and 4E-BP1) and their phosphorylation levels in H9C2 cells in each group were detected by western blot analysis. **p < 0.05 for pairwise comparison. Replicates = 3. Data in panels B and C were analyzed with one-way ANOVA followed by Tukey's multiple comparisons test.

170x100mm (300 x 300 DPI)

Figure 9 Overexpression of PGC1α reduced the damage of BAF60c to H9C2 cells. H9C2 cells with BAF60c overexpression were transfected with PGC1α overexpression plasmid. A. Crystal violet staining was used to detect the surface area of H9C2 cells in two groups ($\times 400$); B. Colony formation assay was used to detect the colony formation ability of H9C2 cells in two groups; C. Flow cytometry was used to detect the apoptosis of H9C2 cells in two groups; D. RT-qPCR was used to detect the expression of indices related to oxidative metabolism and glycolysis of H9C2 cells in two groups; E. RT-qPCR was used to detect the mRNA expression of Hexo2, PFK-M and GAPDH in two groups; F. Relative ATP and MMP levels in H9C2 cells in two groups; G. ROS content in H9C2 cells was detected by DHE fluorescence probe ($\times 400$); H. $[Ca^{2+}]_i$ in H9C2 cells in two groups was detected by Fluo-3/AM fluorescence probe ($\times 400$). ** $p < 0.05$ vs the BAF60c group. Replicates = 3. Data in panels A, C, F, G, and H were analyzed with independent t test, and data in panels D and E were analyzed using two-way ANOVA followed by Sidak's multiple comparisons test.

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Table 1 Primer sequences of RT-qPCR

Primer	Sequence (5'-3')
ANP	F: ACCGAAGATAACAGCCAAATC R: GGGTATTCACACCTCTCAGTG
BNP	F: GAGACAAGAGAGAGCAGGACAC R: ATCCTGCTGCTGCTAATGGATGGA
α -MHC	F: ACTCTTCCTTGTCATCGGGC R: AGGACAAAGCCACTCGCTG
cTnI	F: TCTGAAATTCTGCCCCACCC R: GCCGCTAGAGGTGAGGTATC
β -MHC	F: AGTGACTTGCCAGTTGCGA R: GCCCTACGTTGGGATCTCTG
ssTnI	F: TGTAGAAGTGTATGCAGCAGCC R: AAGACTGTCCGGGGAAAACA
fTnT	F: CGTTCAACTCTCTCCCCAGTC R: GGAGAGAGTTGAACGCCCC
BAF60c	F: GGGGTCTGCCTGTTGACTCG R: ACTATTTGTGGCGAGAGCCG
PGC1 α	F: CTAGCTAGCATGGCTTGGGACATGTGC R: CCGCTCGAGTTACCTGCGCAAGCTTCTC
PPAR α	F: CCGTTTCCACAAGTGCCTGT R: TTCCGAATCTTTCAGATCGTGTT
mCK	F: GGTGTTACCGAATGGCATGG R: CAAGTTACACAGCCTGCCTGA
MCAD	F: CTGCTCGCAGAAATGGCGATGAAA R: CAAAGGCCTTCGCAATAGAGGCAA
CPT-1	F: CTGATGACGGCTATGGTGTTT R: GTGAGGCCAAACAAGGTGATA
UCP3	F: ACTGTTGTGGGAGGCACTTCTGAT R: GCAAGTGCTGCAGTTTCTCC
PDK4	F: TCACTCTCAGTTGGGTACTGT R: TGTGGTGTGACCGAATCCTG
Hexo2	F: GCTAGGGAACCAAACGGTGA R: TCTTCTTTGGGCCAACAGGG
PFK-M	F: AGGCTTTTGGTCTCCACCTG R: GGCAGGTATGGCTGTCTGAT
ALDOLASE	F: CGAGCCCTGGTAAGGATAGG R: GACCGCTCGGAGTGTACTTT
β -actin	F: GTCATTCCAAATATGAGAGATGCGT R: GCTATCACCTCCCCTGTGTG

Note: RT-qPCR, reverse transcription quantitative polymerase chain reaction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; MHC, myosin heavy chain; cTnI, cardiac troponin I; ssTnI, slow skeletal troponin I; fTnT, fast skeletal troponin T; PPAR α , peroxisome proliferator-activated receptor α ; PGC-1 α , PPAR- γ coactivator-1 α ; mCK, mitochondrial creatine kinase; MCAD, medium chain acyl-CoA dehydrogenase; CPT-1, carnitine palmitoyl transferase 1; UCP3, uncoupling protein-3; PDK4, pyruvate dehydrogenase kinase isozyme4; Hexo2,

Hexosaminidase 2; PFK-M phosphofructokinase muscle-specific isoform.

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Table 2 Primary antibodies used in Western blot analysis

<u>Primary</u> antibody	Item No.	Ratio
caspase-3	ab13847	1/500
Bax	ab32503	1/1000
Bcl-2	ab182858	1/2000
BAF60c	ab171075	1/1000
PGC-1 α	ab54481	1/1000
PPAR α	ab24509	5 μ g/mL
p-mTOR	ab109268	1/2000
mTOR	ab2732	1/2000
P-p70S6K	ab59208	1/500
P70S6K	ab32529	1/5000
P-4E-BP1	ab47365	1/500
4E-BP1	ab32024	1/5000
<u>β-actin</u>	<u>ab8227</u>	<u>1/1000</u>

Note: Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X; PPAR α , peroxisome proliferator-activated receptor α ; PGC-1 α , PPAR- γ coactivator-1 α ; mTOR, mammalian target of rapamycin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.