Irisin ameliorates septic cardiomyopathy via inhibiting DRP1-related mitochondrial fission and normalizing the JNK-LATS2 signaling pathway

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Abstract

Irisin plays a protective effect in acute and chronic myocardial damage, but its role in septic cardiomyopathy is unclear. The aim of our study was to explore the *in vivo* and *in vitro* effects of irisin using an LPS-induced septic cardiomyopathy model. Our results demonstrated that irisin treatment attenuated LPS-mediated cardiomyocyte death and myocardial dysfunction. At the molecular level, LPS application was associated with mitochondrial oxidative injury, cardiomyocyte ATP depletion and caspase-related apoptosis activation. In contrast, the irisin treatment sustained mitochondrial function by inhibiting DRP1-related mitochondrial fission and the reactivation of mitochondrial fission impaired the protective action of irisin on inflammation-attacked mitochondria and cardiomyocytes. Additionally, we found that irisin modulated DRP1-related mitochondrial fission through the JNK-LATS2 signaling pathway. JNK activation and/or LATS2 overexpression abolished the beneficial effects of irisin on LPS-mediated mitochondrial stress and cardiomyocyte death. Altogether, our results illustrate that LPS-mediated activation of DRP1-related mitochondrial fission through the JNK-LATS2 pathway participates in the pathogenesis of septic cardiomyopathy. Irisin could be used in the future as an effective therapy for sepsis-induced myocardial depression because it corrects DRP1-related mitochondrial fission and normalizes the JNK-LATS2 signaling pathway.

Keywords Irisin · DRP1-related mitochondrial fission · JNK-LATS2 signaling pathway

Introduction

Sepsis is a life-threatening organ dysfunction caused by imbalances in the body's response to infection. The heart is one of the most vulnerable organs during sepsis development (Botker et al. 2018; Zhao et al. 2019), and the incidence of septic cardiomyopathy is approximately 13.8% (Botker et al. 2018; Kokkinaki et al. 2019). The manifestation of septic cardiomyopathy includes ventricular dilatation, a reduction of the left ventricular ejection fraction, and massive cardiomyocyte death. It has been estimated that more than 20% of patients with sepsis develop severe heart failure, and the mortality rate of these patients is ~70% (Charpentier et al. 2004; Coverstone et al. 2018). Despite the therapeutic advances made with respect to timely diagnosis and effective treatment, sepsis and septic cardiomyopathy remain a leading cause of death (Chrifi et al. 2019; Yang et al. 2018). Therefore, it is very important to investigate the molecular mechanism of septic cardiomyopathy and to identify protective strategies for alleviating cardiomyocyte damage and the findings would clinically benefit patients with septic cardiomyopathy.

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The metabolism and performance of cardiomyocytes require the constant production of ATP by mitochondria (Chen et al. 2018; Pan et al. 2018). Accordingly, mitochondrial dysfunction is the primary cause of cardiomyocyte death and heart failure during sepsis challenge (Pan et al. 2018; Skobowiat et al. 2018). Notably, several researchers have shown that mitochondrial malfunction occurs before the myocardial inflammatory response and cardiomyocyte death (Davidson et al. 2018; Kamura et al. 2018). Several mechanisms have been proposed to explain sepsis-mediated cardiomyocyte mitochondrial death in septic cardiomyopathy, and these include mitochondrial respiratory disorder, calcium overload, mitochondrial autophagy arrest, and mitochondrial membrane damage (Deussen 2018; Huang et al. 2018). Mitochondrial fission has recently been recognized as an upstream signal for mitochondrial homeostasis (Zhou et al. 2017; Zhou et al. 2019). Increased mitochondrial fission promotes the formation of mitochondrial fragmentations and thereby induces mitochondrial damage (Zhou et al. 2018). In cardiac ischemia reperfusion injury, fatty liver disease, diabetic nephropathy, and cancers, active mitochondrial fission augments mitochondrial damage and promotes functional cell death (Moulis et al. 2017; Tong et al. 2016; Zhou et al. 2018). Notably, recent studies have also revealed the key role played by mitochondrial fission in sepsis-related myocardial damage (Dong et al. 2013), but the mechanisms through which several effective drugs control mitochondrial fission and thus attenuate septic cardiomyopathy have not been explored.

Irisin, a newly identified hormone, plays a vital role in regulating human body metabolism and oxidative stress, and several studies have reported the beneficial effects of irisin on cardiovascular disorders (Cheng et al. 2018; Li et al. 2019). In a model of murine ischemia reperfusion injury, irisin attenuates reperfusion-mediated heart damage through SOD1-dependent mitochondrial protection (Wang et al. 2018). In obese mice, irisin reverses the mitochondrial content and thereby suppresses adipocyte differentiation and improves insulin sensitivity (Zhou et al. 2016). In addition, irisin controls mitochondrial thermogenesis in cardiomyoblasts (Xie et al. 2015). Molecular-level investigations have shown that mitochondrial oxidative stress, mitochondrial apoptosis, and mitochondrial metabolism are closely modulated by irisin (Wang et al. 2017; Wang et al. 2018), but the influence of irisin on mitochondrial fission in the setting of septic cardiomyopathy has not been explored.

Previous studies have found that mitochondrial fission is primarily modulated by the JNK pathway. For example, in tongue cancer, cell viability is determined by mitochondrial fission, which is highly affected by the JNK-Fis1 biological axis (Zhou et al. 2019), and in diabetic nephropathy, hyperglycemia-mediated renal injury is regulated by the JNK-MFF-mitochondrial fission pathway (Sheng et al. 2019). In addition, in postinfarction cardiac injury, the JNK-Drp1 pathway is the upstream mediator of mitochondrial

fission and participates in the management of cardiomyocyte death and myocardial fibrosis (Wang and Song 2018). JNK activation significantly increases the expression of large tumor suppressor kinase 2 (LATS2), and this effect is closely associated with the activation of mitochondrial fission in lung cancer (Xie et al. 2019) and myocardial reperfusion injury (Nakamura et al. 2016). In the present study, we questioned whether the JNK pathway and LATS2 are implicated in sepsis-mediated myocardial injury. Altogether, the aim of our study was to determine whether irisin attenuates septic cardiomyopathy by modulating mitochondrial fission through the JNK-LATS2 signaling pathway.

Materials and methods

Animals treatment

Wild type (WT) mice with C57BL/6 background (2-month old) were treated with LPS injection (4 mg/kg) to induce septic cardiomyopathy based on a previous study (Dong et al. 2013). Besides, mice were also injected with irisin (5 mg/L) 24 h before LPS treatment according to a previous study (Li et al. 2018).

Echocardiography

All animals under light anesthesia were conducted by echocardiography (Vevo 2100, a 18–38 MHz transducer), refer to the previous literature (Zhu et al. 2018). Briefly, to ensure that the mitral and aortic valves and the apex were visualized, parasternal long-axis views were obtained and recorded and short-axis views were recorded at the mid-papillary muscle level. In order to calculate the end diastolic and end systolic LV areas, endocardial area tracings were conducted in 2D mode from digital images captured on a cineloop. All measurements were made by a single observer and were averaged over three to five consecutive cardiac cycles (Frandsen and Narayanasamy 2018; Sinha et al. 2018). The reproducibility of measurements was assessed in two sets of baseline measurements in 10 randomly selected cardiomyocytes, and the repeated measure variability did not exceed 65% (Kanwar et al. 2018).

Sample collection

After the experiment, mice were anesthetized with phenobarbital sodium and blood samples were obtained from abdominal aorta, and sera were collected and stored for analysis. Heart tissues were collected (Zhang et al. 2018). After weighting on an electronic balance, some tissues were fixed in 10% formaldehyde for histological analysis, and some fresh specimens were immediately put into liquid nitrogen, and then transferred to a -80°C freezer (Edwards et al. 2018).

Cardiomyocyte isolation and treatment

Cardiomyocytes were isolated based on a recent report (Zhou et al. 2017). LPS at 4 µg/ml was used to incubate with cardiomyocytes for 24 h *in vitro*. Besides, cardiomyocytes were treated with irisin (20 nmol/L) 24 h before LPS stress. LATS2 adenovirus (ad-LATS2), purchased from Shanghai GenePharma Co. (Shanghai, China), was transfected into cardiomyocytes based on a previous study. Anisomycin (Ani, 10 µM, Selleck Chemicals, Houston, TX, USA) was used to activate JNK, and FCCP (5 µM, Selleck Chemicals, Houston, TX, USA) was used to modulate mitochondrial fission. Cardiomyocyte mechanical properties were measured via a SoftEdge MyoCam system (IonOptix, Milton, MA) as previously described (Fukumoto et al. 2018).

Transmission electron microscope observation

Sections from each group were fixed with 2.5% glutaraldehyde in 0.1-mol/L PBS (pH = 7.4) at 37 °C for 90 min, post-fixed in 1% osmium tetroxide for 30 min, and washed with PBS. Subsequently, the cells were progressively dehydrated in a 10% graded series of 50–100% ethanol and propylene oxide and embedded in Epon 812 resin (Maria et al. 2018). The blocks were cut into ultrathin section (50–150-µm thick) with a vibratome (UC7; Leica EM, Solms, Germany), then stained with saturated uranyl acetate and lead citrate. Finally, we observed the morphology of cardiomyocytes using the transmission electron microscope (H-7500, Hitachi, Japan) as previously described (Faughnan et al. 2019).

MitoTracker red staining

Mitochondrial length was assessed with MitoTracker red staining. After necessary treatment, cardiomyocytes were washed three times with PBS. Then, MitoTracker red was added to the phenol red-free medium at the final concentration of 20 nM and maintained for 30 min at 37 °C in darkness. Nuclei of cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (Farber et al. 2018). Fluorescent images were visualized and recorded using $\times 100$ oil immersion on a Zeiss fluorescence microscope. The average length of mitochondria was calculated according to a previous study (Li et al. 2018).

Enzyme-linked immunosorbent assays

Levels of inflammatory factors, such as serum CK-MB, troponin T, TNF- α , IL-6, C3a, and LDH, were measured with ELISA kits (Bio-Swamp Immunoassay R&D Center, Shanghai, China), according to the manufacturers' directions. OD at 450 nm was measured with a microplate reader. A standard curve was generated by plotting OD value versus

standard concentration (Meyer and Leuschner 2018). The curve equation and *r* value were calculated and used to determine their concentrations (Darden et al. 2019).

Mitochondrial membrane potential measurements and mitochondrial ROS detection

A JC-1 probe (Beyotime, China, Cat. No: C2006) was used to observe mitochondrial potential (Rusnati et al. 2019) and MitoSOX red mitochondrial superoxide indicator (Molecular Probes, USA) was used to quantify mitochondrial reactive oxygen species (ROS) production according to a previous study (Dong et al. 2018).

TUNEL staining

The paraffin sections obtained from the above experiment were dewaxed with xylene for 10 min, again 10 min with fresh xylene, and dehydrated through a serial alcohol gradient. Twenty µg/mL DNase-free protease K was added into tissue slides and incubated at 20–37 °C for 15–30 min, followed by a PBS wash for three times. Then, 50-µL TUNEL solution was added into tissue slides and incubated at 37 °C for 60 min in the dark. After washing by PBS for three times, tissue slides were treated with anti-fluorescence quenching agent and then observed by fluorescence microscopy at excitation wavelength of 450–500 nm and emission wavelength of 515–565 nm (Li et al. 2018).

Measurement of intracellular ATP

Intracellular ATP levels in cells were measured using a firefly luciferase-based ATP assay kit (Beyotime, China) in accordance with the manufacturer's instructions. Briefly, cardiomyocytes were lysed and subjected to centrifugation at $10,000 \times g$ for 10 min at 4 °C. An equal volume (50 µl) of supernatant from each group was mixed with 50-µl luciferin/luciferase reagent to catalyze the light production from ATP and luciferin. Chemiluminescence was measured with a fluorescence microplate reader. Intracellular levels of ATP were normalized to protein concentrations (Edwards et al. 2018).

Western blot

Proteins were extracted from the heart tissues and cardiomyocytes using a RIPA lysis buffer. Samples (50 µg) were carried out with 10–12% sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene fluoride (PVDF) membranes (Shi et al. 2018), which were blocked with 5% skim milk for 1 h at room temperature. The membranes were washed in TBST for 3 times and were incubated with one of the following primary antibodies: Bcl2 (1:1000, Cell Signaling Technology, #3498),

Bax (1:1000, Cell Signaling Technology, #2772), caspase-9 (1:1000, Cell Signaling Technology, #9504), survivin (1:1000, Cell Signaling Technology, #2808), Drp1 (1:1000, Abcam, #ab56788), Fis1 (1:1000, Abcam, #ab71498), Opa1 (1:1000, Abcam, #ab42364), Mfn2 (1:1000, Abcam, #ab56889), TNF- α (1:1000, Abcam, #ab66671), IL-6 (1:1000, Abcam, #ab7737), C3a (1:1000, Abcam, #ab48581), complex III subunit core (CIII-core2, 1:1000, Invitrogen, #459220), complex II (CII-30, 1:1000, Abcam, #ab110410), complex IV subunit II (CIV-II, 1:1000, Abcam, #ab110268), t-JNK (1:1000; Cell Signaling Technology, #4672), p-JNK (1:1000; Cell Signaling Technology, #9251).

Confocal imaging

After endogenous peroxidase activity was quenched for 10 min in phosphate-buffered saline (PBS) containing 3% H₂O₂, it was heated in antigen retrieval solution (ethylenediaminetetraacetic acid, pH 8.0) at 90 °C for 10 min, chilled in water, and then immersed for 5 min in PBS. Sections were incubated with primary antibodies: cyt-c (1:1000; Abcam; #ab90529). Confocal imaging was obtained under a Zeiss LSM 880 Confocal microscope (Gonzalez et al. 2018).

Data analysis

Data were statistically treated by analysis of variance using SPSS (IBM, Armonk, NY, USA), followed by Student Newman Keul's post hoc test. The data were expressed as mean $\pm$ standard deviation. $p < 0.05$ was considered statistically significant.

Results

Irisin attenuates LPS-mediated myocardial depression

Septic cardiomyopathy in mice was established by LPS injection, and the mice were also treated with irisin to observe its protective effects. First, the alterations in inflammation factors were observed by ELISA. As shown in Fig. 1A–C, the levels of TNF- α , IL-6, and C3a were rapidly increased in response to the LPS treatment compared with the levels found in the control group. These alterations were also observed in the myocardium by western blotting (Fig. 1D–G). Interestingly, irisin treatment attenuated the elevation in inflammation factors, as demonstrated by ELISA (Fig. 1A–C) and western blotting (Fig. 1D–G). Moreover, the concentrations of cardiac markers, such as LDH, CK-MB, and troponin T, were also upregulated in LPS-treated mice and were reduced to near-normal levels with the irisin treatment (Fig. 1H–J). This result indicated the cardioprotective effects of irisin in the setting of septic

cardiomyopathy. The alterations in cardiac function were observed by echocardiography. As shown in Fig. 1K, L, LPS application reduced the fractional shortening (FS) and left ventricular ejection fraction (LVEF) compared with the levels found for the control group. However, the irisin treatment sustained cardiac function under LPS stress (Fig. 1K, L). The structural changes in LPS-treated myocardium were then observed by electron microscopy. As shown in Fig. 1M, the LPS treatment mediated myocardial fiber breakage, myocardium dissolution, and Z-line disappearance; and these alterations could be reversed by the irisin treatment. Altogether, the above-described data indicate that irisin attenuates LPS-mediated myocardial injury.

Irisin improves cardiomyocyte survival in LPS-treated hearts

Massive cardiomyocyte death is associated with myocardial depression induced by LPS stress. TUNEL staining showed that the number of TUNEL-positive cells was rapidly increased in response to the LPS treatment (Fig. 2A, B). However, the irisin treatment reduced the proportion of TUNEL-positive cells, indicating the anti-apoptotic effect of irisin on LPS-treated hearts. In addition, we also isolated cardiomyocytes from LPS-treated hearts and measured various single-cardiomyocyte mechanical parameters according to a previous study (Jin et al. 2018). As shown in Fig. 2C–H, the LPS treatment and/or irisin supplementation had no influence on the cell length of resting cardiomyocytes compared with the control group. Interestingly, the LPS treatment significantly inhibited the peak shortening (PS) and maximal velocity of shortening/relengthening ($\pm$ dL/dt), and the time to peak shortening (TPS) and time to 90% relengthening (TR₉₀) were increased by the LPS treatment compared with the control levels (Fig. 2C–H). In contrast, the irisin treatment reversed the PS/ $\pm$ dL/dt and reduced both the TPS and TR₉₀. Therefore, the above-described data indicated that irisin maintained cardiomyocyte function under LPS stress. Primary cardiomyocytes were also isolated and treated with LPS and/or irisin, and their viability was then assessed using a CCK-8 assay. As shown in Fig. 2I, the LPS treatment reduced the viability of cardiomyocytes compared with the control group, and this decrease could be reversed by the irisin treatment. Cardiomyocyte death in response to the LPS treatment was also assessed by measuring caspase-3 activity. As shown in Fig. 2J, the LPS treatment elevated the activity of caspase-3 compared with that found for the control group, and this effect was abolished by irisin application. Altogether, the above-described data indicate that irisin supplementation maintains cardiomyocyte viability under LPS stress.

Fig. 1 Irisin treatment attenuates LPS-mediated myocardial damage. (A–C) Mice were treated with LPS injection and irisin treatment. Then, the content of inflammatory factors was measured via ELISA. (D–G) Proteins were isolated from LPS-treated heart tissues and then, the expression of inflammatory factors was determined via western blots. (H–J)

ELISA was used to evaluate the alterations of cardiac damage markers such as LDH, CK-MB, and Troponin T. (K, L) Cardiac function was determined via echocardiogram. (M) Electron microscope was used to observe the alterations of myocardium. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. LPS group

Irisin sustains mitochondrial function in LPS-treated cardiomyocytes

Cardiomyocyte function and viability are closely linked to mitochondrial function (Zhou et al. 2018). Experiments were then performed to determine whether irisin attenuates LPS-mediated myocardial injury by modulating mitochondrial function. The mitochondrial membrane potential determines the mitochondrial energy metabolism and ATP supply. Using a JC-1 probe, we found that the mitochondrial membrane potential was significantly depressed by the LPS treatment,

as evidenced by a decrease in red fluorescence and an increase in green fluorescence (Fig. 3A–B). Interestingly, the irisin treatment reversed the mitochondrial membrane potential. At the molecular level, the cellular ATP content was regulated by the mitochondrial membrane potential. However, due to the LPS-mediated reduction in the mitochondrial potential, the ATP content was also inhibited in the LPS group (Fig. 3C) and this effect was reversed by the irisin treatment. Moreover, the expression of the mitochondrial respiratory complex was also downregulated by the LPS treatment (Fig. 3D–G) and this alteration was reversed by the irisin treatment. Altogether, the

Fig. 2 Irisin treatment sustains cardiomyocyte function in the presence of LPS stress. (A, B) TUNEL staining was used to observe the cell death in response to irisin treatment and/or LPS stress. (C–H) The contractile properties of cardiomyocytes in WT mice in the context of LPS-mediated inflammation injury. +dL/dt is the maximal velocity of shortening. –dL/dt is the maximal velocity of relengthening. TPS is the

time to peak shortening. TR₉₀ is the time to 90% relengthening. (I) CCK8 assay was used to observe cell viability in response to LPS stress and/or irisin treatment. (J) ELISA assay was used to evaluate the caspase-3 activity in cardiomyocyte. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. LPS group

above-described data indicated that the irisin treatment reversed the LPS-mediated changes in mitochondrial function in cardiomyocytes.

Irisin supplementation abolishes LPS-mediated mitochondrial apoptosis in cardiomyocytes

Excessive mitochondrial damage initiates mitochondrial apoptosis, which is characterized by mitochondrial oxidative stress, the release of cyt-c, and the upregulation of mitochondrial pro-apoptotic proteins (Zhou et al. 2018). A flow cytometric analysis showed that the LPS treatment elevated mitochondrial ROS production (Fig. 4A, B), and this effect was negated by the irisin treatment. This result indicated the anti-oxidative effect of irisin on LPS-treated cardiomyocytes. In addition, the release of cyt-c was assessed through an immunofluorescence assay. As shown in Fig. 4C, D, compared with the control group, the LPS treatment elevated the nuclear content of cyt-c, as demonstrated by the appearance of a greater amount of cyt-c in the nucleus. However, the irisin treatment

prevented the LPS-mediated release of cyt-c. Excessive cyt-c release upregulates mitochondrial pro-apoptotic factors, and a western blot analysis demonstrated that Bax and caspase-9 expression was rapidly increased in response to the LPS treatment (Fig. 4E–I). In contrast, the levels of anti-apoptotic proteins, such as Bcl-2 and survivin, were also downregulated after exposure to LPS (Fig. 4E–I). Interestingly, the irisin treatment reversed the LPS-induced increases in the expression of anti-apoptotic factors and the LPS-induced reductions in the levels of pro-apoptotic proteins (Fig. 4E–I). Overall, the above-described data illustrate that LPS-activated mitochondrial apoptosis can be inhibited by the irisin treatment.

Irisin inhibits DRP1-related mitochondrial fission under the LPS treatment

Mitochondrial fission is considered an upstream mediator of mitochondrial function and apoptosis (Zhou et al. 2018). A western blotting analysis demonstrated that mitochondrial fission-related proteins, such as DRP1 and Fis1, were

Fig. 3 Mitochondrial dysfunction is improved by irisin in the presence of LPS. (A, B) Mitochondrial membrane potential was determined via JC-1 staining. Red fluorescence indicates the normal mitochondrial membrane potential whereas green fluorescence indicates the damaged mitochondrial membrane potential. (C) ATP production was measured

to reflect the mitochondrial energy metabolism. (D–G) Proteins were isolated from cells and then, the expression of mitochondria respiratory complex was evaluated via western blotting. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. LPS group

significantly increased in response to the LPS treatment (Fig. 5A–E). In contrast, the levels of anti-fission proteins in cardiomyocytes were clearly inhibited by LPS (Fig. 5A–E). These data verified the promotive effect of LPS on mitochondrial fission. Notably, the irisin treatment prevented the LPS-mediated upregulation of mitochondrial fission factors, as evidenced by decreases in DRP1 and Fis1 expressions (Fig. 5A–E). Moreover, the LPS-mediated changes in the content of anti-fission proteins were reversed to near-normal levels by the irisin treatment (Fig. 5A–E). This finding was further supported through an immunofluorescence assay. As shown in Fig. 5F, G, the control mitochondria presented a long fusiform shape with an average length ranging from 8.9 to 9.6 μ m, and treatment with LPS mediated mitochondrial division into several fragments with a reduced length of ~ 4.3 μ m. In addition, the appearance of mitochondrial fragmentation in the LPS group was accompanied by an increase in the DRP1 levels (Fig. 5F, G), which confirmed that the LPS treatment activated DRP1-related mitochondrial fission. Interestingly, the irisin

treatment reversed the LPS-induced changes in mitochondrial length and prevented DRP1 upregulation (Fig. 5F, G), which suggests that irisin exerts an inhibitory effect on mitochondrial fission under LPS stress.

Mitochondrial fission reactivation abolishes the protective effects of irisin on cardiomyocyte viability and mitochondrial function

To investigate whether irisin protects cardiomyocytes and mitochondria against LPS-mediated injury through the inhibition of mitochondrial fission, gain-of-function assays of mitochondrial fission were performed using FCCP, a specific mitochondrial fission agonist, and cardiomyocyte viability and mitochondrial function were then re-measured. First, cardiomyocyte viability, as assessed through an analysis of LDH release, was significantly inhibited by the LPS treatment, and this effect was reversed to near-normal levels by the irisin treatment (Fig. 6A). Interestingly, the application of FCCP

Fig. 4 Mitochondrial apoptosis is initiated by LPS, which is effectively inhibited by irisin treatment. (A, B) ROS production was measured via flow cytometry. (C, D) Immunofluorescence assay for cyt-c. The fluorescence intensity of nuclear cyt-c was measured to reflect the

activation of mitochondrial apoptosis. The nuclear expression of cyt-c was recorded. (E–I) Proteins were isolated from cells and then, the expression of mitochondria apoptosis proteins was evaluated via western blotting. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. LPS group

abolished the protective effects of irisin on cardiomyocyte viability (Fig. 6A). In addition, caspase-3 activity in cardiomyocytes was also augmented by the LPS treatment (Fig. 6B). Interestingly, irisin prevented the activation of caspase-3 (Fig. 6B) and this effect was negated by FCCP, an activator of mitochondrial fission. The function of the mitochondria was also evaluated, and the results showed that ATP production was rapidly downregulated by the LPS treatment (Fig. 6C). In addition, the irisin treatment reversed the LPS-induced downregulation of ATP (Fig. 6C) and this effect was abrogated by FCCP treatment. In addition, via immunofluorescence, we also found that the LPS-mediated release of cyt-c could be inhibited by the irisin treatment and FCCP treatment reinduced the translocation of cyt-c into the nucleus (Fig. 6D, E). In addition, caspase-9 activity was also increased in

response to the LPS treatment (Fig. 6F). Interestingly, the irisin treatment prevented the activation of caspase-9 (Fig. 6F) and this effect was abolished through the activation of mitochondrial fission. Altogether, the above-described data indicated that irisin sustains cardiomyocyte viability and mitochondrial function under LPS stress by suppressing mitochondrial fission.

Irisin modulates DRP1-related mitochondrial fission via the JNK-LATS2 signaling pathway

Although we found that irisin-mediated cardioprotection through repressing mitochondrial fission, the molecular mechanism through which irisin modulates mitochondrial fission is unknown. Previous studies have identified the JNK pathway

Fig. 5 Mitochondrial fission is modulated by irisin in the presence of LPS-mediated inflammation injury. (A–E) Proteins were isolated from cells and then, the expression of mitochondria fission-related proteins was evaluated via western blotting. (F, G) Co-immunofluorescence assay for mitochondria and Drp1. The expression of Drp1 was determined and the average length of mitochondria was measured. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. LPS group

and LATS2 as the primary upstream mediators of mitochondrial fission (Xie et al. 2019). In the present study, a western blot analysis demonstrated that p-JNK and LATS2 expressions were significantly elevated in response to the LPS treatment (Fig. 7A–C) and this upregulation is indicative of the LPS-mediated activation of the JNK pathway and LATS2. Interestingly, the irisin treatment inhibited the expressions of p-JNK and LATS2 (Fig. 7A–C), which suggested that irisin exerts inhibitory effects on the JNK pathway and LATS2. Notably, re-activation of the JNK pathway using anisomycin (Ani) reversed the irisin-mediated effects on LATS2 expression in cardiomyocytes (Fig. 7A–C), which confirmed that the JNK-LATS2 pathway was modulated by irisin under LPS stress.

Experiments were then performed to verify the involvement of the JNK-LATS2 signaling pathway in irisin-mediated DRP1-related mitochondrial fission (Liu et al. 2018). As shown in Fig. 7D–F, compared with the control group, the LPS treatment elevated the expression of Drp1, indicative of the activation of Drp1-related mitochondrial fission. Interestingly, the irisin treatment inhibited the upregulation of Drp1 in the presence of LPS. Interestingly, overexpression of LATS2 via transfection of LATS2 adenovirus (ad-LATS2) abolished the regulatory effect of irisin on Drp1 depression. Besides, an immunofluorescence assay was used to further demonstrate the alterations of Drp1-related mitochondrial fission in response to JNK activation, LATS2 overexpression in the presence of the irisin treatment (Souza et al. 2018). As shown in Fig. 7G, H, compared with the control group, the LPS treatment elevated the fluorescence intensity of DRP1, which was accompanied with a decline in the average length of mitochondria. Notably, the irisin treatment inhibited Drp1 upregulation and reversed mitochondrial length in the presence of LPS stress. Interestingly, activation

of JNK using Ani and/or overexpression of LATS2 via transfecting ad-LATS2 abolished the inhibitory effect of irisin on DRP1 and mitochondrial fission. Overall, the above-described data indicate that the JNK-LATS2 pathway is modulated by irisin and contributes to the DRP1-related management of mitochondrial fission under LPS stress.

Discussion

The primary finding of our study is that cardiac cardiomyopathy is associated with the initiation of DRP1-related mitochondrial fission due to JNK-LATS2 signaling pathway activation. As shown in the study, uncontrolled mitochondrial fission mediated mitochondrial damage and even augmented mitochondria-related apoptosis signals. Subsequently, excessive mitochondrial stress evoked cardiomyocyte death and thereby hindered cardiomyocyte contraction and reduced cardiac function. Interestingly, the irisin treatment effectively inhibited mitochondrial damage by correcting DRP1-related mitochondrial fission. Mechanistically, irisin application inhibited the JNK-LATS2 signaling pathway, and this effect suppressed DRP1 expression and subsequently repressed mitochondrial division. Due to its inhibitory effects on mitochondrial fission, irisin sustained mitochondrial function, maintained cardiomyocyte viability, and improved cardiac function in the setting of LPS-induced septic cardiomyopathy. Overall, although many studies have reported the beneficial effects of irisin on mitochondria and reperfused hearts, our study provides new data to explain the cardioprotective actions of irisin in septic cardiomyocytes. This study constitutes the first exploration of the relationship between irisin and mitochondrial fission in the cardiovascular system, and the

Fig. 6 Irisin attenuates LPS-mediated mitochondrial damage and cell death via inhibiting mitochondrial fission. (A) LDH release assay was used to evaluate the cell death in response to irisin treatment. FCCP, an activator of mitochondrial fission, was used to activate mitochondrial fission in irisin-treated cells. (B) ELISA assay was used to evaluate the caspase-3 activity in response to LPS treatment. FCCP was used to recall mitochondrial fission in irisin-treated cells. (C) ATP production was measured to reflect the mitochondrial energy metabolism. (D, E) Immunofluorescence assay for cyt-c. The nuclear expression of cyt-c was measured to reflect the cyt-c translocation. (F) Caspase-9 activity was determined to quantify the activation of mitochondrial apoptosis.

* $p < 0.05$ vs. control group;

$p < 0.05$ vs. LPS group;

@ $p < 0.05$ vs. LPS + irisin group

findings might pave a new road for the development of therapies for septic cardiomyopathy.

The mitochondria play critical roles in the pathogenesis of septic cardiomyopathy through the modulation of inflammation signals (Matkovich et al. 2017). In the present study, exposure to an inflammatory environment caused mitochondria to generate excessive ROS. Based on a previous study, ROS accumulation impairs protein structure and function and thereby worsens several cellular physiological processes (Flynn et al. 2010), such as glucose oxidative phosphorylation, lipid metabolism, and gene copy/transcription. In response to mitochondrial oxidative stress, various cellular anti-oxidative factors, such as SOD, GSH, and GPX, are activated to correct the redox balance. However, our results

indicated that the mitochondrial membrane potential was reduced if the intrinsic anti-oxidative factors alone were not sufficient to attenuate mitochondrial damage. The reduction in the mitochondrial potential limited the production of ATP and thereby reduced cardiomyocyte contraction. More severely, a loss in the mitochondrial membrane potential is also considered an early marker of mitochondrial apoptosis. The release of mitochondrial pro-apoptotic factors was followed by a decrease in the mitochondrial potential, which ultimately resulted in the activation of members of the caspase family. Thus, protection of mitochondrial function is vital to sustain cardiomyocyte viability.

In the present study, we found that mitochondrial fission was the core regulatory signal for mitochondrial homeostasis.

Fig. 7 Irisin controlled mitochondrial fission in a manner dependent on the JNK-LATS2-Drp1 signaling pathways. (A–C) Proteins were isolated from cells and then, the expressions of JNK and LATS2 were evaluated via western blotting. To activate the JNK pathway, anisomycin (Ani) was added into irisin-treated cells. (D–F) Proteins were isolated from cells and then, the expressions of JNK and LATS2 were evaluated via western blotting. To reverse the expression of LATS2, adenovirus-loaded LATS2 (ad-LATS2) was transfected into cells to reverse LATS2 expression. (G, H) Co-immunofluorescence assay for mitochondria and Drp1. The expression of Drp1 was determined and the average length of mitochondria was measured. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. LPS group; @ $p < 0.05$ vs. LPS + irisin group

In fact, many studies have reported the role of mitochondrial fission in cardiac physiology and pathophysiology (Adaniya et al. 2019). For example, in myocardial senescence, DRP1-mediated mitochondrial hyperfusion promotes cardiomyocyte dysfunction by inducing oxidative stress (Nishimura et al. 2018), and in myocardial ischemia reperfusion injury, the inhibition of DRP1-related mitochondrial fission via mi-RNA-140 promotes cardiomyocyte survival by repressing mitochondria-related cardiomyocyte death (Yang et al. 2019). Moreover, in postinfarction cardiac injury, Sirt3-mediated cardioprotection is dependent on the inhibition of mitochondrial fission through the AMPK-DRP1 pathway (Liu et al. 2019). In the present study, we found that mitochondrial fission induced by the irisin treatment sustained mitochondrial function and improved cardiomyocyte viability in the setting of LPS-mediated septic cardiomyopathy. This finding implicates mitochondrial fission as a candidate inducer of myocardial dysfunction, on one hand. On the other hand, irisin could be used as an effective approach to correct the excessive mitochondrial fission and thus reduce septic cardiomyopathy in clinical practice.

At the molecular level, mitochondrial fission is controlled by DRP1 and the activity of Drp1 is affected by the JNK pathway. For example, in postinfarction cardiac injury,

increased JNK activity is positively linked to the expression of DRP1 (Wang and Song 2018). Interestingly, in rectal cancer, the JNK pathway modifies the activity of DRP1 by inducing the posttranscriptional phosphorylation of DRP1 at Ser616 (Li et al. 2017). Notably, in heart failure, the JNK pathway also affects DRP1 expression through the ERK signaling pathway (Huang et al. 2018). In the present study, we found that the JNK pathway is activated by the LPS-induced inflammation microenvironment and that increased JNK expression is followed by DRP1 upregulation, which indicated that the JNK pathway could be considered the upstream mediator of DRP1. However, the detailed molecular mechanisms through which JNK modulates DRP1 expression were not investigated and additional studies are needed to answer this question.

Altogether, our results demonstrate the beneficial effects of irisin on septic cardiomyocytes. The irisin treatment attenuated sepsis-mediated myocardial depression and cardiomyocyte death by inhibiting DRP1-related mitochondrial fission through inhibition of the JNK-LATS2 signaling pathway. These data reveal a crucial function for irisin in the maintenance of cardiac function, which suggests that JNK-LATS2-mediated mitochondrial fission could be a novel target for the treatment of septic cardiomyopathy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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