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Original Article

FOXO1 regulates NLRP3 inflammasome proteins in LPS-induced cardiotoxicity

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Abstract: Objective: Forkhead box protein O1 (FOXO1) has been shown to regulate multiple proteins in various cardiovascular disease processes. However, the effect of FOXO1 on lipopolysaccharide (LPS)-induced cardiotoxicity remains unknown. The aim of this study was to explore the impact of FOXO1 on LPS-induced cardiotoxicity. Methods: Rat-derived H9c2 cells were subjected to LPS, and the manipulation of FOXO1 was achieved through overexpression and knockdown using the adeno-associated virus system and siRNA, respectively. Western blotting and quantitative real-time polymerase chain reaction were utilized to examine the inhibitory effect of FOXO1. Cell viability was examined utilizing Cell Counting Kit-8 assay. The changes of apoptosis were examined utilizing Annexin V-FITC/PI method. The levels of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-18, and tumor necrosis factor-α in the H9c2 cells were measured using ELISA kits. Reactive oxygen species (ROS) generation was quantified using the 2'-7'dichlorofluorescin diacetate assay kit. Results: In H9c2 cells treated with LPS, FOXO1 expression was downregulated in a dose-dependent and time-dependent manner. Overexpression of FOXO1 attenuated LPS-induced apoptosis, oxidative stress injury, and cardiomyocyte inflammation, while FOXO1 inhibition aggravated these processes. Additionally, FOXO1 was found to regulate LPS-related myocardial injury by downregulating the expression of NLR family pyrin domain-containing 3 (NLRP3). Conclusion: FOXO1 overexpression attenuated apoptosis, ROS generation, and inflammation, whereas FOXO1 inhibition aggravated LPS-induced cardiomyocyte injury via the NLRP3 inflammasome signaling pathway.

Keywords: Lipopolysaccharide, FOXO1, NLRP3, H9c2 cells, cardiomyopathy, inflammation

Introduction

Sepsis, which is caused by life-threatening infections, is a complex systemic inflammatory response syndrome [1]. The high mortality rate of sepsis is caused by the development of multiple organ failure, of which sepsis-induced cardiomyopathy is a very common cause [2-4]. Previous studies have reported that approximately 43% of patients with sepsis have cardiac injury and positive serum troponin levels [5, 6]. In addition, cardiac dysfunction has been confirmed to be a major factor leading to mortality and morbidity in these patients [7].

Forkhead box protein O1 (FOXO1) is a key transcription factor involved in the regulation of a variety of physiological processes, including apoptosis, fibrosis, and cardiac hypertrophy [8-11]. It was confirmed that overexpression of FOXO1 could aggravate aging-related cardiac dysfunction by promoting muscle cell death [12]. The RNA binding protein Quaking (QKI) could also promote nitrosative stress and endoplasmic reticulum stress by upregulating FOXO1 expression in mice [13]. Moreover, FOXO1 expression was found to be downregulated in the lipopolysaccharide (LPS)-induced acute kidney injury model, and overexpression of FOXO1 via adeno-associated virus delivery could enhance renal function and reduce mitochondrial damage [14]. Chalise et al. previously reported a relationship between FOXO1 and LPS in the heart [15]. Furthermore, FOXO1 is highly expressed cardiac tissue, and the expression levels of genes and proteins change with

the progression of dilated cardiomyopathy [16]. In terms of signaling pathways, the three main kinases, Akt, Erk1/2 and Ikk, mediate the phosphorylation, inactivation and degradation of FOXO [17]. Previously studies have reported the mechanism of LPS-induced cardiotoxicity, involving apoptosis, inflammation and ROS generation [18, 19]. Nevertheless, the role of FOXO1 in LPS-induced cardiomyocyte injury remains unclear.

The aim of this study was to investigate the changes of FOXO1 expression in H9c2 cells after LPS stimulation and the effects of FOXO1 intervention on LPS-related cardiomyocyte injury. The underlying mechanism by which FOXO1 regulated LPS-induced cardiotoxicity was also explored.

Materials and methods

Cell culture and treatment

Rat cardiomyocyte-derived H9c2 cells (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1% penicillin/streptomycin at 37°C in a 5% CO_2 incubator. LPS (Sigma, St. Louis, MO, USA) at concentrations of 1, 5, 10, 50, or 100 μg/mL was added to culture medium. The cells were treated with 10 μg/mL LPS for 0.25, 0.5, 1, 2, and 3 days. siRNA of FOXO1 (si-FOXO1) and negative control (si-NC), pcDNA3.1-FOXO1, and Ad-NC (GenePharma Co., Shanghai, China) were used for the transfection experiment. After transfection with si-NC, si-FOXO1, pcDNA3.1-GFP, or pcDNA3.1-FOXO1 using Lipofectamine 3000 for 6-8 h and subsequent medium change, the cells were treated with 10 μg/mL LPS for 1 day after 24 hours. This study was approved by the Ethics Committee of Tianjin Fifth Central Hospital.

Cell viability

Cell Counting Kit (CCK)-8 assay (Dojindo, Kumamoto, Japan) was conducted as described previously [18]. Briefly, H9c2 cells were inoculated into 96-well plates (1×10^4 cells/well) overnight. After incubation under different conditions, CCK-8 (10 μL) was supplemented, and cells were cultivated for additional 2 h. The $OD_{450\ nm}$ (optical density) was detected by a microplate reader (Bio-Rad, Hercules, CA, USA).

Apoptosis

The changes in apoptosis were examined utilizing Annexin V-FITC/PI Kit (JIANCHENG Biotech Co., Ltd., Shanghai, China). Following LPS stimulation, the cells were collected and washed 3 times with phosphate-buffered saline. Subsequently, the cells were incubated in dark with 10 μL of Annexin V-FITC and 5 μL of PI for 15 min. The apoptosis rate was determined by FACSCalibur flow cytometer (BD Biosciences). The levels of apoptosis were determined by quantifying the number of cells positively stained solely with Annexin V, following the previously described method [21].

Western blotting

Protein was extracted from H9c2 cells using RIPA buffer. Samples (30 μg/lane) were loaded on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membrane, which were blocked with 5% skimmed milk at room temperature for 1 h, rinsed with PBST three times, and cultivated with the primary antibodies FOXO1, (#2880, 1:1000, Cell Signaling Technology), NLR family pyrin domain-containing 3 (NLRP3) (ab214185, 1:1000, Abcam), and GAPDH (ab9485, 1:1000, Abcam) at 4°C overnight. Afterwards, the membranes were probed with horseradish peroxidase (HRP)-labeled secondary antibody and visualized using enhanced chemiluminescence (ECL) reagents in a ChemiDoc Touch chemiluminescence detection system (Bio-Rad, Hercules, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

TRIzol (Invitrogen, Carlsbad, CA, USA) was used to lyse the cells and extract total RNA. Next, RNA was reversely transcribed into cDNA using a cDNA Synthesis Kit (Roche, USA). Briefly, 10 μL of the extracted DNA was tested in a final reaction volume of 25 μl containing 12.5 μL of ExTaq 2 × probe mix. Green Master Mix (YEASEN, Shanghai, China) was used to perform qRT-PCR using the specific primer sequences (**Table 1**). The real-time PCR was performed with the following cycling program: 5 min at 95°C, followed by 45 cycles of 30 s at 55°C and 20 s at 65°C on a BioRad CFX 96 thermocycler (Hercules, California, USA). In each run, non-template controls were included.

Table 1. List of primer sequences generated for qPCR

Gene	Primer sequences (5'-3')	
	Forward	Reverse
NLRP3	GTGGAGATCCTAGGTTTCTCTG	CAGGATCTCATTCTCTTGGATC
TNF-α	AGCATGATCCGAGATGTGGAA	TAGACAGAAGAGCGTGGTGGC
FOXO1	CTTGGAGAAGGGGATGTGC	TGTTGGTGATGAGAGAAGGTTG
IL-1β	CAGGTCGCTCAGGGTCACA	CAGAGGCAAGGAGGAAACACA
IL-18	AAAGATAGCCAGCCTAGAGGTATG	GATCTATCCCCCAATTCATCCT
GAPHD	TATGTCGTGGAGTCTACTGGT	GAGTTGTCATATTTCTCGTGG

NLRP3: NLR family pyrin domain-containing 3; TNF-α: tumor necrosis factor-α; FOXO1: Forkhead box protein O1; IL-1β: interleukin-1β; IL-18: interleukin-18; GAPHD: glyceraldehyde 3-phosphate dehydrogenase.

The mRNA expressions were calculated using the $2^{-\Delta\Delta Ct}$ method.

Enzyme-linked immunosorbent assay (ELISA)

The levels of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-18, and tumor necrosis factor (TNF)-α in the H9c2 cells were detected using ELISA kits (Bio-Swamp Immunoassay R&D Center, Shanghai, China) following the relevant instructions. The $OD_{450\ nm}$ was obtained by a microplate reader.

Reactive oxygen species (ROS) generation

The H9c2 cells were inoculated in 6-well plates at a density of 5×10^5 cells/well. After treatment with LPS for 1 d, ROS generation was quantified using the 2'-7'dichlorofluorescin diacetate (DCFH-DA) assay kit (Ab238535, Abcam, Cambridge, United Kingdom) following the manufacturer's instructions. Briefly, 50 μL of samples or hydrogen peroxide standards were added into a black 96-well plate. Catalyst (50 μL) was added into each well, incubated for 5 min at room temperature, then DCFH solution (100 μL) was added and incubated for 30 min in dark at room temperature. Fluorescence was read at 480 nm/530 nm (excitation/emission).

Statistical analysis

The data were analyzed using Prism 7.0 (GraphPad Software Inc., CA, USA) and expressed as mean ± standard deviation (SD). Differences between two groups were analyzed using an unpaired two-sided t-test, whereas differences among three or more groups were examined utilizing one-way analysis of variance (ANOVA) with Tukey's post hoc test. $P < 0.05$ indicated statistical significance.

Results

LPS treatment decreased cell viability in a time-dependent and dose-dependent manner

First, LPS-induced cardiotoxicity was established by stimulating the H9c2 cells with various concentrations of LPS (1, 5, 10, 50, and 100 μg/mL) for 1 day. LPS treatment significantly decreased cell viability in a dose-dependent manner compared with the control group (**Figure 1A**). Next, the H9c2 cells were stimulated with 10 μg/mL LPS for 0.25, 0.5, 1, 2, and 3 d. As expected, cell viability was decreased in a time-dependent manner (**Figure 1B**). Thus, in the subsequent experiments, the H9c2 cells were treated with 10 μg/mL LPS for 1 d to establish cardiomyocyte injury.

LPS increased apoptosis, ROS generation, and inflammation

The apoptosis rate of the LPS group was significantly higher than that in the control group (**Figure 2A**). The ROS generation was also increased after LPS treatment (**Figure 2B**). Additionally, the levels of pro-inflammatory cytokines were markedly increased in the LPS group, as determined using ELISA (**Figure 2C-F**). These results indicated that LPS stimulation induced obvious inflammatory and apoptotic injury in the H9c2 cells.

LPS decreased FOXO1 expression in the H9c2 cells

To determine the association of FOXO1 levels with LPS, FOXO1 protein and mRNA levels were determined using Western blotting and qRT-PCR, respectively. After stimulating the H9c2 cells with different concentrations of LPS (1, 5, 10, 50, or 100 μg/mL) for 1 day, FOXO1 protein and mRNA levels were significantly downregulated in a dose-dependent manner compared with those in the control group (**Figure 3A** and **3B**). Similarly, FOXO1 expression was markedly downregulated in a time-dependent manner after LPS stimulation for 6, 12, 24, 48, and 72 h (**Figure 3C** and **3D**). These findings suggested that FOXO1 played a role in LPS-induced cardiotoxicity.

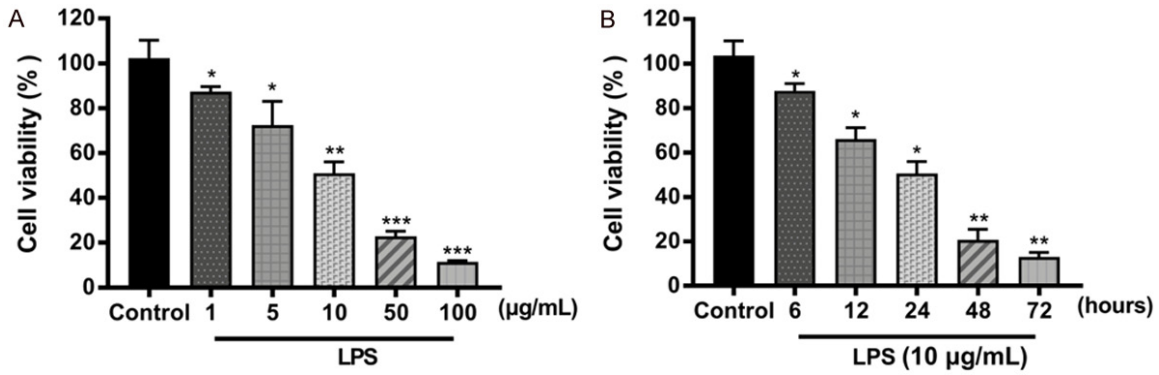

Figure 1. LPS treatment decreases cell viability in H9c2 cells. A. Cell viability was examined using the CCK-8 assay after the cells were treated with various concentrations of LPS (1, 5, 10, 50, and 100 μg/mL) for 24 h. B. Cell viability was determined with 10 μg/mL LPS for 6, 12, 24, and 48 h. n=12, *P < 0.05, **P < 0.01, ***P < 0.001, compared with the control group. LPS: lipopolysaccharide.

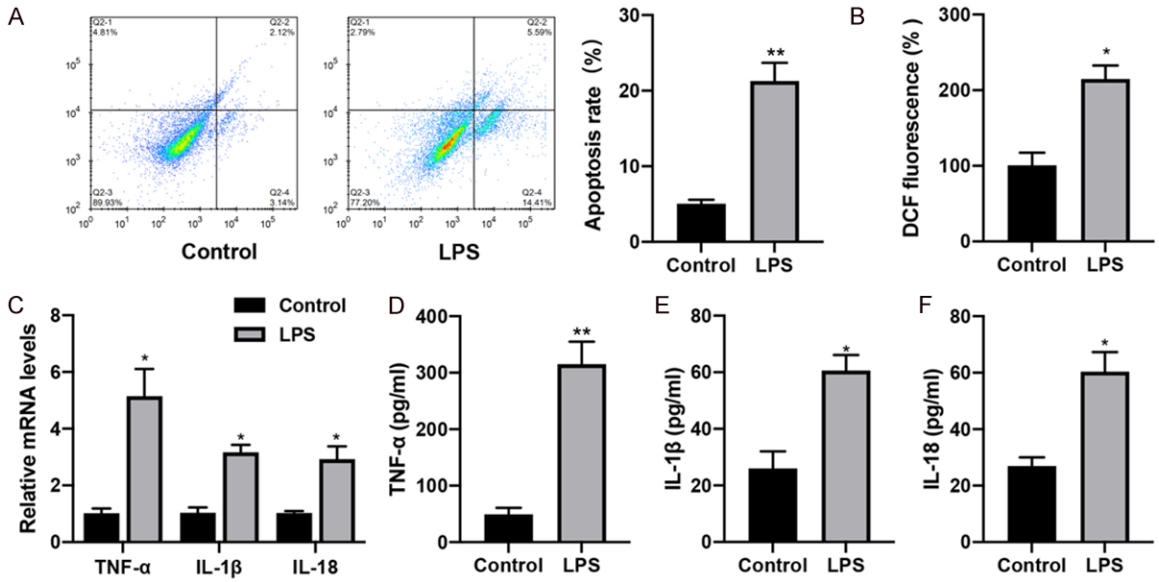

Figure 2. Effects of LPS on apoptosis, oxidative stress, and inflammation. (A) The apoptosis rate was measured using the Annexin V-FITC/PI kit after treating the cells with LPS for 24 h. (B) ROS generation was determined using the ROS assay. The mRNA (C) and protein levels of TNF-α (D), IL-1β (E), and IL-18 (F) were determined using qRT-PCR and ELISA, respectively. n=6, *P < 0.05, **P < 0.01, compared with the control group. LPS: lipopolysaccharide; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; IL-18: interleukin-18; qRT-PCR: quantitative real-time polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay.

FOXO1 inhibited LPS-induced apoptosis

The effects of FOXO1 on LPS-induced cardiotoxicity were explored. pcDNA3.1-FOXO1 or si-FOXO1 was transfected into the H9c2 cells to overexpress or knockdown FOXO1, respectively. Western blotting and qRT-PCR were utilized to detect the transfection efficiency of pcDNA3.1-FOXO1 and si-FOXO1. In the H9c2 cells, pcDNA3.1-FOXO1 significantly increased the FOXO1 protein and mRNA levels, while si-FOXO1 significantly decreased those levels (**Figure 4A** and **4B**). In addition, compared with that in the control group, the viability of the H9c2 cells with overexpressed FOXO1 was significantly increased after LPS stimulation, whereas si-FOXO1 aggravated the LPS-induced decline in cell viability (**Figure 4C**). Furthermore, the apoptosis rate of H9c2 cells treated with LPS showed a decrease upon FOXO1 overexpression and an increase after FOXO1 inhibition (**Figure 4D**). These results provide evidence

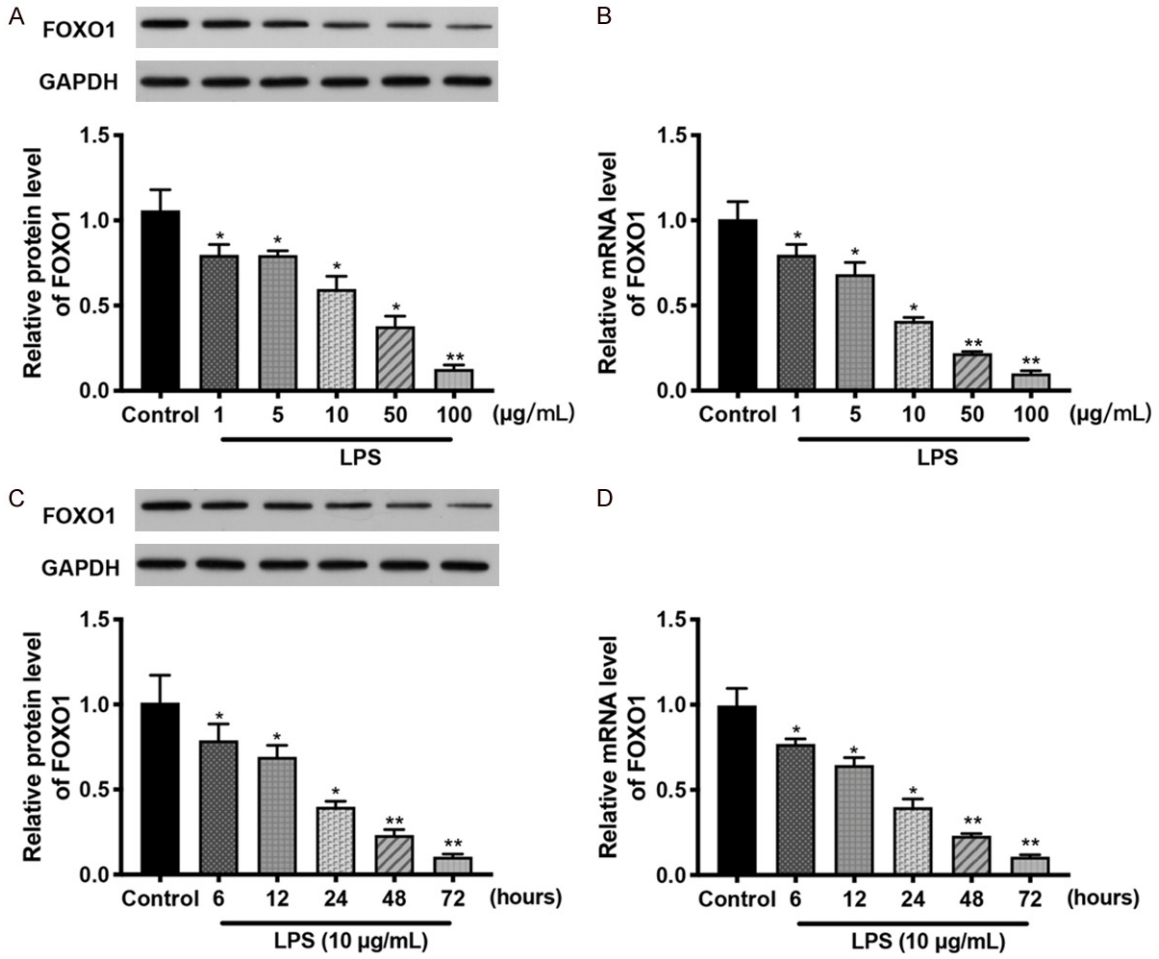

Figure 3. FOXO1 expression in H9c2 cells is downregulated after LPS treatment. A, B. The FOXO1 protein and mRNA levels in the cells treated with 1, 5, 10, 50, and 100 μg/mL LPS for 24 h were determined. C. FOXO1 protein levels after stimulation with 10 μg/mL LPS for 6, 12, 24, and 48 h. D. FOXO1 mRNA levels after stimulation with 10 μg/mL LPS for 6, 12, 24, and 48 h. n=6, *P < 0.05, **P < 0.01, compared with the control group. FOXO1: Forkhead box protein O1; LPS: lipopolysaccharide.

that FOXO1 overexpression attenuated LPS-induced apoptosis of the H9c2 cells.

FOXO1 suppressed myocardial inflammation accumulation and LPS-induced ROS generation

The impact of FOXO1 on LPS-induced oxidative damage was investigated. Myocardial ROS generation was decreased in LPS-treated H9c2 cells with overexpressed FOXO1. Conversely, si-FOXO1 further aggravated LPS-induced oxidative damage (**Figure 5**). The effects of FOXO1 on LPS-induced inflammation were explored by studying the production of pro-inflammatory cytokines after FOXO1 overexpression. After LPS stimulation, si-FOXO1 further upregulated the mRNA levels of the pro-inflammatory cytokines (TNF-α, IL-1β, and IL-18), whereas the overexpression of FOXO1 alleviated these effects (**Figure 6A-C**). In addition, changes in the cytokine protein levels, as measured by ELISA, were consistent with the mRNA levels, as assayed by qRT-PCR (**Figure 6D-F**). These data suggested that FOXO1 inhibited LPS-induced oxidative damage and inflammatory responses in the H9c2 cells.

FOXO1 altered NLRP3 expression

Previous studies determined that FOXO1 decreased NLRP3 expression during inflammation [19, 20]. qRT-PCR and Western blotting were utilized to quantify the NLRP3 mRNA and protein levels, respectively, in H9c2 cells with FOXO1 overexpression or FOXO1 knockdown to

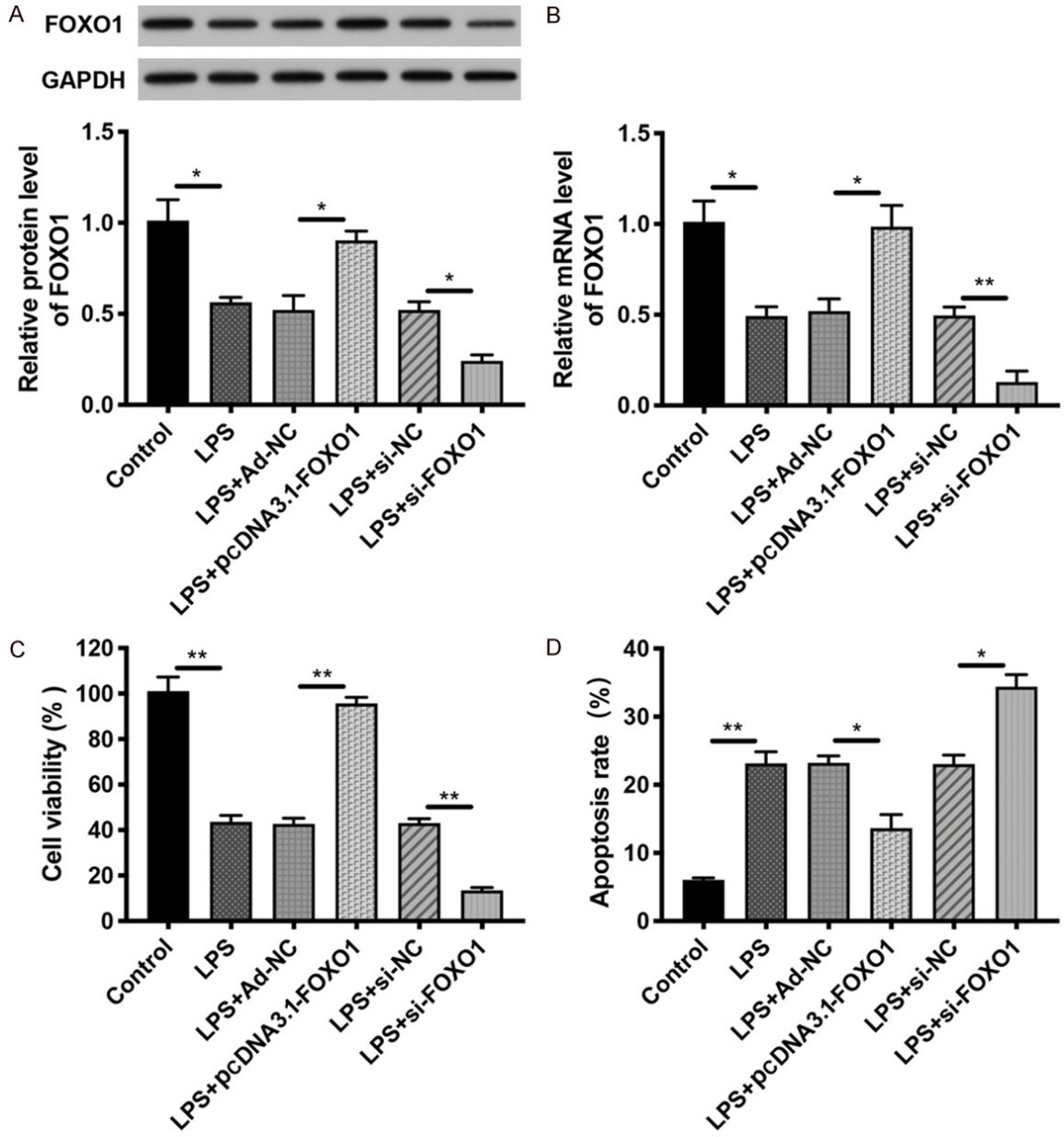

Figure 4. FOXO1 attenuates LPS-induced decreased cell viability and increased apoptosis. (A) Representative Western blot images of FOXO1 levels in the H9c2 cells after transfection with pcDNA3.1-FOXO1 or Ad-NC, si-FOXO1 or si-NC after LPS treatment. (B) FOXO1 mRNA expression was measured using PCR. Cell viability (C) and the apoptosis rate (D) after FOXO1 overexpression or knockdown. n=6, *P < 0.05, **P < 0.01, compared with the matched control. FOXO1: Forkhead box protein O1; LPS: lipopolysaccharide; PCR: polymerase chain reaction.

verify whether FOXO1 regulated the inflammatory response by altering the expression of NLRP3 after LPS stimulation. The NLRP3 protein and mRNA levels were markedly upregulated in the H9c2 cells after LPS stimulation, whereas FOXO1 overexpression downregulated the expression of NLRP3 (**Figure 7A** and **7B**). Furthermore, the downregulation of FOXO1 resulted in a significant upregulation of NLRP3 expression. These results indicate that the NLRP3 might be a target of FOXO1 in the context of LPS-induced cardiotoxicity. To investigate the effect of NLRP3 in FOXO1-mediated LPS-induced inflammation, NLRP3 was overexpressed or silenced in LPS-treated cells with FOXO1 overexpression or silencing (**Figure 8A**). ELISA was performed to assess the regulation of inflammatory cytokines. The results clearly

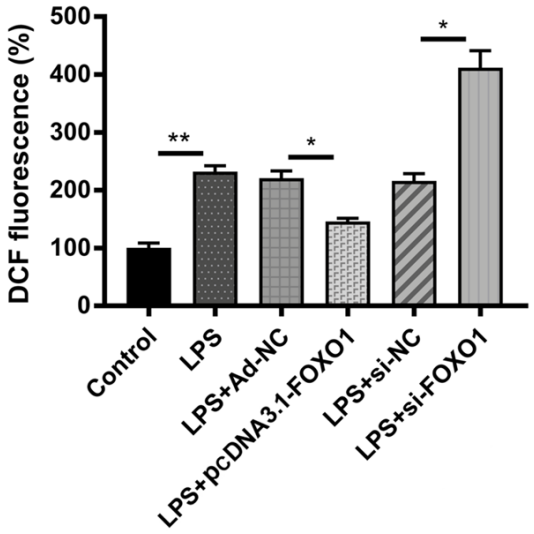

Figure 5. FOXO1 attenuates LPS-induced oxidative stress. ROS generation was examined using the DCFH-DA assay in the Control, LPS, LPS + Ad-NC, LPS + pcDNA3.1-FOXO1, LPS + si-NC, and LPS + si-FOXO1 groups. n=6, *P < 0.05, **P < 0.01, compared with the matched control. FOXO1: Forkhead box protein O1; LPS: lipopolysaccharide; ROS: reactive oxygen species; DCFH-DA: 2'-7'dichlorofluorescin diacetate.

demonstrated that NLRP3 counteracted the inhibitory effect of FOXO1 on inflammation (**Figure 8B-D**).

Discussion

Studies have revealed that excessive apoptosis, oxidative stress, and inflammation play a critical role in cardiac dysfunction during sepsis [3, 21]. This study was the first to report the protective effect of FOXO1 on sepsis-induced cardiomyopathy. The in vitro functional studies of FOXO1 overexpression and knockdown in LPS-induced cardiotoxicity suggested that FOXO1 negatively regulated sepsis-induced cardiomyopathy and prevented oxidative damage and systemic inflammation by inhibiting NLRP3 expression.

FOXO1 is an important regulator of oxidative stress response, cell proliferation, cell death, and metabolism [22]. Deletion of FOXO1 could lead to cardiac hypertrophy and consequently heart failure [23]. Moreover, FOXO1 activity after insulin resistance could increase the apoptosis rate and disrupt energy metabolism by upregulating the levels of β-MHC in the heart [11]. LPS is an outer membrane component of Gram-negative bacteria, which triggers myocardial abnormalities in sepsis [24]. After exposure to LPS, the expression of FOXO1 was decreased in the kidney, and the overexpression of FOXO1 alleviated LPS-induced injury of renal tubular epithelial cells [14]. This study also found that LPS could decrease FOXO1 expression in a time-dependent and dose-dependent manner. In addition, the overexpression of FOXO1 in the H9c2 cells could promote cell viability after LPS treatment. These data indicated that FOXO1 played a crucial role in LPS-related cardiac injury.

Increased ROS generation is an essential mechanism of sepsis-induced cardiomyopathy [25-28]. Excessive ROS generation can induce mitochondrial oxidative damage and apoptosis in the heart. FOXO1 has been shown to upregulate the levels of antioxidant genes in oxidative stress response, increase ROS scavenging activity, and reduce DNA damage and apoptosis [13]. FOXO1 has also been confirmed to be a key regulator of cell longevity by decreasing ROS generation [12]. A previous study also showed that SIRT1/FOXO1a/SOD2 signaling attenuated mitochondrial damage by inhibiting NLRP3 inflammasome [29]. Even though ROS plays a vital role in LPS-induced inflammatory response, low levels of ROS have been reported to be necessary for the immune system to maintain health. However, it was also found that inhibiting FOXO1-NLRP3 inflammasome was advantageous in mitigating smoke inhalation-induced lung injury [30]. ROS interactions with NLRP3 inflammasomes participate in regulating the immune inflammation responses. Studies have reported the relationship between ROS and NLRP3 inflammasomes. (1) ROS generation partly depends on activation of NLRP3 inflammasomes. (2) ROS-mediated DAMPs are involved in activating NLRP3 inflammasomes to increase inflammation. (3) ROS-induced NLRP3 components may be important factors in triggering apoptosis of immune cells [31]. In this study, inhibition of FOXO1 further increased ROS generation as well as NLRP3 upregulation after LPS treatment, whereas overexpression of FOXO1 reduced ROS-related cell injury and apoptosis, as well as NLRP3 expression. These findings suggested that FOXO1 could protect cardiomyocytes from LPS-induced injury by inhibiting ROS generation and cell apoptosis. However, further investigation is warranted to delve deeper into this mechanism.

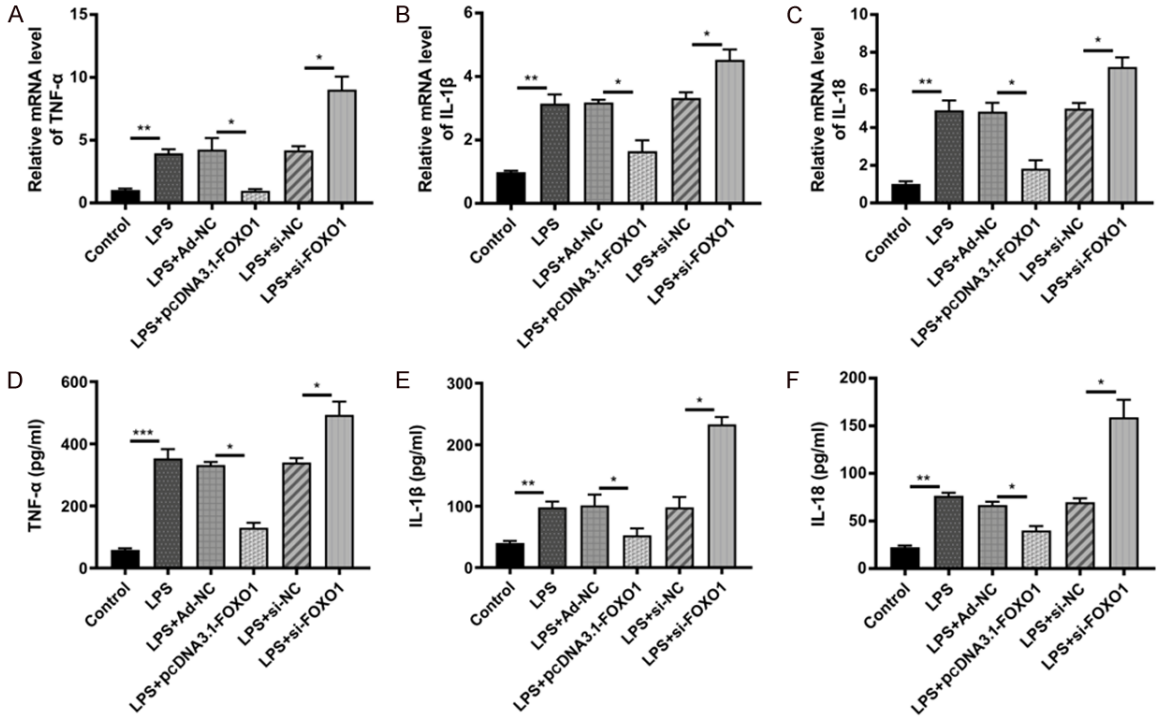

Figure 6. FOXO1 suppresses LPS-related inflammation. A-C. The mRNA levels of pro-inflammatory cytokines in the H9c2 cells, including TNF-α, IL-1β, and IL-18, after transfection with pcDNA3.1-FOXO1 or Ad-NC, si-FOXO1 or si-NC after LPS treatment, as determined using qRT-PCR. D-F. The levels of inflammatory cytokines, including TNF-α, IL-1β, and IL-18, in H9c2 cells after transfection with pcDNA3.1-FOXO1 or Ad-NC, si-FOXO1 or si-NC after LPS treatment, as determined using ELISA. n=6, *P < 0.05, **P < 0.01, ***P < 0.001, compared with the matched control. FOXO1: Forkhead box protein O1; LPS: lipopolysaccharide; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; IL-18: interleukin-18; qRT-PCR: quantitative real-time polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay.

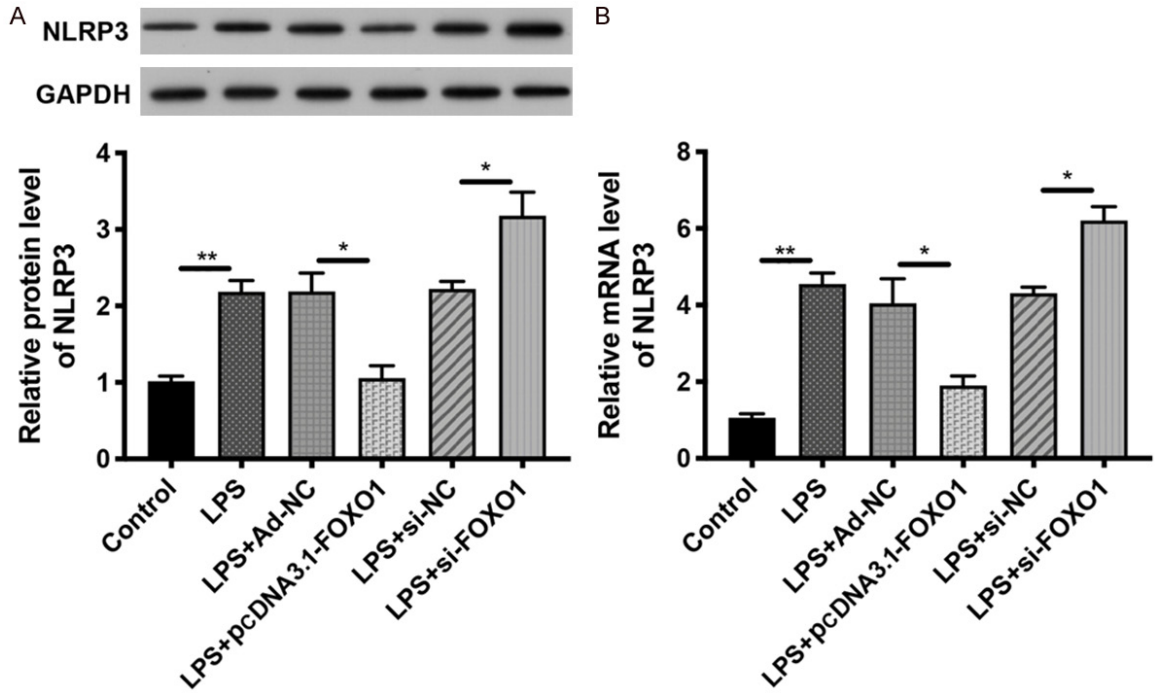

Figure 7. FOXO1 decreases NLRP3 expression. A. Representative Western blot images of NLRP3 expression of the H9c2 cells in the Control, LPS, LPS + Ad-NC, LPS + pcDNA3.1-FOXO1, LPS + si-NC, and LPS + si-FOXO1 groups. B. mRNA levels of NLRP3, as determined using qRT-PCR. *P < 0.05, **P < 0.01, compared with the matched control. FOXO1: Forkhead box protein O1; NLRP3: NLR family pyrin domain-containing 3; LPS: lipopolysaccharide; qRT-PCR: quantitative real-time polymerase chain reaction.

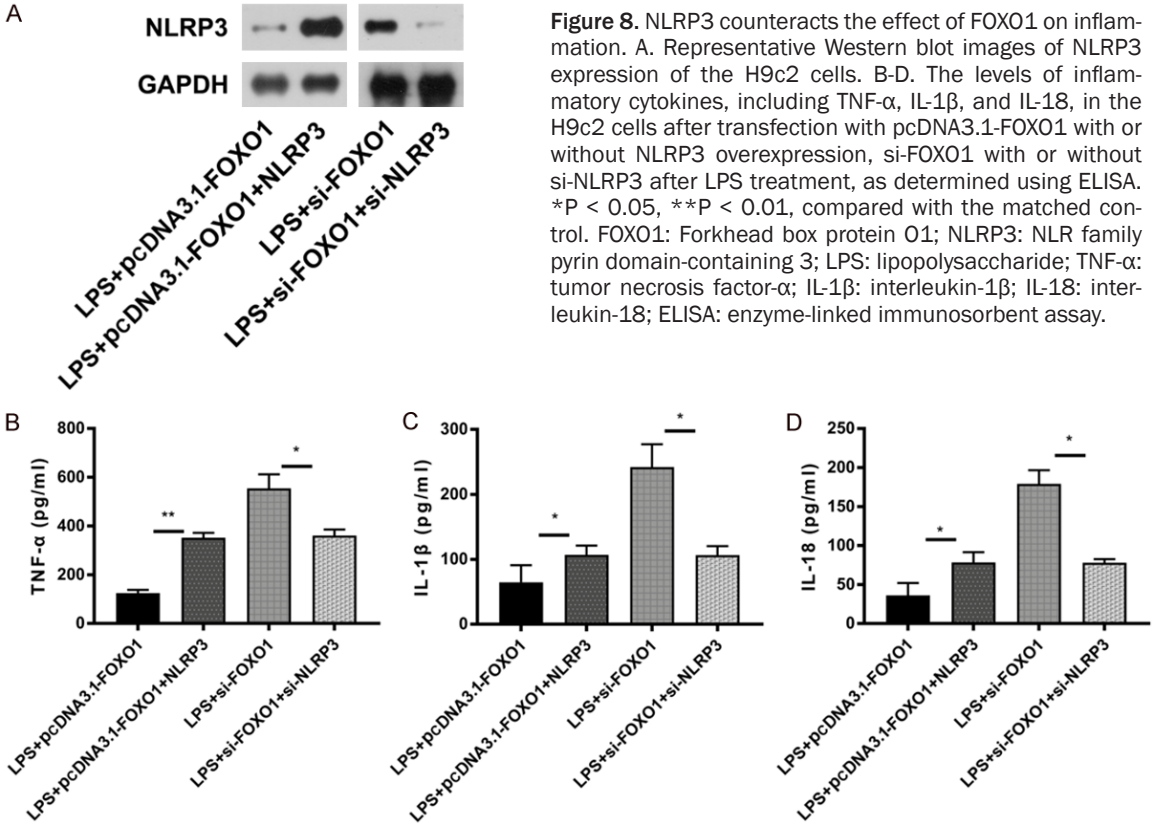

Figure 8. NLRP3 counteracts the effect of FOXO1 on inflammation. A. Representative Western blot images of NLRP3 expression of the H9c2 cells. B-D. The levels of inflammatory cytokines, including TNF-α, IL-1β, and IL-18, in the H9c2 cells after transfection with pcDNA3.1-FOXO1 with or without NLRP3 overexpression, si-FOXO1 with or without si-NLRP3 after LPS treatment, as determined using ELISA. *P < 0.05, **P < 0.01, compared with the matched control. FOXO1: Forkhead box protein O1; NLRP3: NLR family pyrin domain-containing 3; LPS: lipopolysaccharide; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; IL-18: interleukin-18; ELISA: enzyme-linked immunosorbent assay.

Inflammation also plays a significant role in sepsis-induced cardiomyopathy. Several molecular mechanisms, such as the formation of the NLRP3 inflammasome and the activity of the NF-κB pathway, have been proposed to explain the influence of inflammation on LPS-related injury [32-34]. The intervention of the NLRP3 inflammasome or NF-κB pathway decreased the levels of inflammation-associated cytokines in LPS-induced cardiac dysfunction [35, 36]. The activation of NLRP3 increased the serum levels of IL-1β and IL-18. The role of FOXO1 in regulating NLRP3 during liver injury has been demonstrated [19, 37, 38]. In the present study, it was observed that FOXO1 knockdown promoted NLRP3 expression and the release of pro-inflammatory cytokines. These data indicated that FOXO1 aggravated LPS-related inflammation in the H9c2 cells, partly by regulating NLRP3 activity.

This study also has certain limitations. First, the H9c2 cell line was used in vitro, which is the most widely used cell line in previous studies because of its similarity to primary cardiomyocytes in vitro. However, it cannot fully reproduce the characteristics of primary cardiomyocytes. Therefore, further experiments are needed to validate the present results.

In conclusion, FOXO1 downregulation promoted LPS-induced myocardial injury in the H9c2 cells by upregulating NLRP3 expression, whereas FOXO1 overexpression attenuated these effects. Therefore, FOXO1 may be a promising target for the treatment of sepsis-induced cardiomyopathy.

Disclosure of conflict of interest

None.

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References

[1] Cecconi M, Evans L, Levy M and Rhodes A. Sepsis and septic shock. Lancet 2018; 392: 75-87.

[2] Lin H, Wang W, Lee M, Meng Q and Ren H. Current status of septic cardiomyopathy: basic science and clinical progress. Front Pharmacol 2020; 11: 210.

[3] Martin L, Derwall M, Al Zoubi S, Zechendorf E, Reuter DA, Thiemermann C and Schuerholz T. The septic heart: current understanding of molecular mechanisms and clinical implications. Chest 2019; 155: 427-437.

[4] Lelubre C and Vincent JL. Mechanisms and treatment of organ failure in sepsis. Nat Rev Nephrol 2018; 14: 417-427.

[5] Takasu O, Gaut JP, Watanabe E, To K, Fagley RE, Sato B, Jarman S, Efimov IR, Janks DL, Srivastava A, Bhayani SB, Drewry A, Swanson PE and Hotchkiss RS. Mechanisms of cardiac and renal dysfunction in patients dying of sepsis. Am J Respir Crit Care Med 2013; 187: 509-17.

[6] Fernandes CJ Jr, Akamine N and Knobel E. Cardiac troponin: a new serum marker of myocardial injury in sepsis. Intensive Care Med 1999; 25: 1165-8.

[7] Maheshwari K, Nathanson BH, Munson SH, Khangulov V, Stevens M, Badani H, Khanna AK and Sessler DI. The relationship between ICU hypotension and in-hospital mortality and morbidity in septic patients. Intensive Care Med 2018; 44: 857-867.

[8] Xin Z, Ma Z, Hu W, Jiang S, Yang Z, Li T, Chen F, Jia G and Yang Y. FOXO1/3: potential suppressors of fibrosis. Ageing Res Rev 2018; 41: 42-52.

[9] Yang M, Lin Y, Wang Y and Wang Y. High-glucose induces cardiac myocytes apoptosis through Foxo1/GRK2 signaling pathway. Biochem Biophys Res Commun 2019; 513: 154-158.

[10] Yu W, Chen C and Cheng J. The role and molecular mechanism of FoxO1 in mediating cardiac hypertrophy. ESC Heart Fail 2020; 7: 3497-3504.

[11] Qi Y, Zhu Q, Zhang K, Thomas C, Wu Y, Kumar R, Baker KM, Xu Z, Chen S and Guo S. Activation of Foxo1 by insulin resistance promotes cardiac dysfunction and β-myosin heavy chain gene expression. Circ Heart Fail 2015; 8: 198-208.

[12] Spurthi KM, Sarikhani M, Mishra S, Desingu PA, Yadav S, Rao S, Maity S, Tamta AK, Kumar S, Majumdar S, Jain A, Raghuraman A, Khan D, Singh I, Samuel RJ, Ramachandra SG, Nandi D and Sundaresan NR. Toll-like receptor 2 deficiency hyperactivates the FoxO1 transcription factor and induces aging-associated cardiac dysfunction in mice. J Biol Chem 2018; 293: 13073-13089.

[13] Guo W, Jiang T, Lian C, Wang H, Zheng Q and Ma H. QKI deficiency promotes FoxO1 mediated nitrosative stress and endoplasmic reticulum stress contributing to increased vulnerability to ischemic injury in diabetic heart. J Mol Cell Cardiol 2014; 75: 131-140.

[14] Zhang M, Dong W, Li Z, Xiao Z, Xie Z, Ye Z, Liu S, Li R, Chen Y, Zhang L, Wang M, Liang H, Baihetiyaer R, Apaer R, Dong Z and Liang X. Effect of Forkhead box O1 in renal tubular epithelial cells on endotoxin-induced acute kidney injury. Am J Physiol Renal Physiol 2021; 320: F262-F272.

[15] Chalise U, Becirovic-Agic M, Konfrst SR, Rodriguez-Paar JR, Cook LM and Lindsey ML. MMP-12 polarizes neutrophil signalome towards an apoptotic signature. J Proteomics 2022; 264: 104636.

[16] Han R, Huang H, Xia W, Liu J, Luo H, Tang J and Xia Z. Perspectives for forkhead box transcription factors in diabetic cardiomyopathy: their therapeutic potential and possible effects of salvianolic acids. Front Cardiovasc Med 2022; 9: 951597.

[17] Coomans de Brachène A and Demoulin JB. FOXO transcription factors in cancer development and therapy. Cell Mol Life Sci 2016; 73: 1159-1172.

[18] Li Y, Liu X, Du A, Zhu X and Yu B. miR-203 accelerates apoptosis and inflammation induced by LPS via targeting NFIL3 in cardiomyocytes. J Cell Biochem 2019; 120: 6605-6613.

[19] Miyauchi T, Uchida Y, Kadono K, Hirao H, Kawasoe J, Watanabe T, Ueda S, Okajima H, Terajima H and Uemoto S. Up-regulation of FOXO1 and reduced inflammation by β-hydroxybutyric acid are essential diet restriction benefits against liver injury. Proc Natl Acad Sci U S A 2019; 116: 13533-13542.

[20] Wang Q, Hu J, Liu Y, Li J, Liu B, Li M and Lou S. Aerobic exercise improves synaptic-related proteins of diabetic rats by inhibiting FOXO1/NF-κB/NLRP3 inflammatory signaling pathway and ameliorating PI3K/Akt insulin signaling pathway. J Mol Neurosci 2019; 69: 28-38.

[21] Jiang W, Li W, Hu X, Hu R, Li B and Lan L. CTRP1 prevents sepsis-induced cardiomyopathy via Sirt1-dependent pathways. Free Radic Biol Med 2020; 152: 810-820.

[22] Ferdous A, Wang ZV, Luo Y, Li DL, Luo X, Schiattarella GG, Altamirano F, May HI, Battiprolu PK, Nguyen A, Rothermel BA, Lavandero S, Gillette TG and Hill JA. FoxO1-Dio2 signaling axis governs cardiomyocyte thyroid hormone metabolism and hypertrophic growth. Nat Commun 2020; 11: 2551.
[23] Puthanveetil P, Wan A and Rodrigues B. FoxO1 is crucial for sustaining cardiomyocyte metabolism and cell survival. Cardiovasc Res 2013; 97: 393-403.
[24] Luo Y, Fan C, Yang M, Dong M, Bucala R, Pei Z, Zhang Y and Ren J. CD74 knockout protects against LPS-induced myocardial contractile dysfunction through AMPK-Skp2-SUV39H1-mediated demethylation of BCLB. Br J Pharmacol 2020; 177: 1881-1897.
[25] Hwang HJ, Kim JW, Chung HS, Seo JA, Kim SG, Kim NH, Choi KM, Baik SH and Yoo HJ. Knockdown of sestrin2 increases lipopolysaccharide-induced oxidative stress, apoptosis, and fibrotic reactions in H9c2 cells and heart tissues of mice via an AMPK-dependent mechanism. Mediators Inflamm 2018; 2018: 6209140.
[26] Zhang N, Feng H, Liao HH, Chen S, Yang Z, Deng W and Tang QZ. Myricetin attenuated LPS induced cardiac injury in vivo and in vitro. Phytother Res 2018; 32: 459-470.
[27] Zhang X, Wang X, Fan M, Tu F, Yang K, Ha T, Liu L, Kalbfleisch J, Williams D and Li C. Endothelial HSPA12B exerts protection against sepsis-induced severe cardiomyopathy via suppression of adhesion molecule expression by miR-126. Front Immunol 2020; 11: 566.
[28] Huang J, Peng W, Zheng Y, Hao H, Li S, Yao Y, Ding Y, Zhang J, Lyu J and Zeng Q. Upregulation of UCP2 expression protects against LPS-induced oxidative stress and apoptosis in cardiomyocytes. Oxid Med Cell Longev 2019; 2019: 2758262.
[29] Li L, Wang H, Zhao S, Zhao Y, Chen Y, Zhang J, Wang C, Sun N and Fan H. Paeoniflorin ameliorates lipopolysaccharide-induced acute liver injury by inhibiting oxidative stress and inflammation via SIRT1/FOXO1a/SOD2 signaling in rats. Phytother Res 2022; 36: 2558-2571.
[30] Fu Z, Jiang Z, Guo G, Liao X, Liu M and Xiong Z. rhKGF-2 attenuates smoke inhalation lung injury of rats via activating PI3K/Akt/Nrf2 and repressing FoxO1-NLRP3 inflammasome. Front Pharmacol 2021; 12: 641308.
[31] Zhao S, Chen F, Yin Q, Wang D, Han W and Zhang Y. Reactive oxygen species interact with NLRP3 inflammasomes and are involved in the inflammation of sepsis: from mechanism to treatment of progression. Front Physiol 2020; 11: 571810.
[32] Zhong L, Zhou XL, Liu YS, Wang YM, Ma F, Guo BL, Yan ZQ and Zhang QY. Estrogen receptor α mediates the effects of notoginsenoside R1 on endotoxin-induced inflammatory and apoptotic responses in H9c2 cardiomyocytes. Mol Med Rep 2015; 12: 119-126.
[33] Zheng Y, Li S, Hu R, Cheng F and Zhang L. GFI-1 protects against lipopolysaccharide-induced inflammatory responses and apoptosis by inhibition of the NF-κB/TNF-α pathway in H9c2 cells. Inflammation 2020; 43: 74-84.
[34] Liu Y, Yang W, Sun X, Xie L, Yang Y, Sang M and Jiao R. SS31 ameliorates sepsis-induced heart injury by inhibiting oxidative stress and inflammation. Inflammation 2019; 42: 2170-2180.
[35] Wang R, Dong Z, Lan X, Liao Z and Chen M. Sweroside alleviated LPS-induced inflammation via SIRT1 mediating NF-κB and FOXO1 signaling pathways in RAW264.7 cells. Molecules 2019; 24: 872.
[36] Zhou L, Su X, Li B, Chu C, Sun H, Zhang N, Han B, Li C, Zou B, Niu Y and Zhang R. PM2.5 exposure impairs sperm quality through testicular damage dependent on NALP3 inflammasome and miR-183/96/182 cluster targeting FOXO1 in mouse. Ecotoxicol Environ Saf 2019; 169: 551-563.
[37] Zhang Y, Zhu P, Wu X, Yuan T, Su Z, Chen S, Zhou Y and Tao WA. Microcystin-LR induces NLRP3 inflammasome activation via FOXO1 phosphorylation, resulting in interleukin-1β secretion and pyroptosis in hepatocytes. Toxicol Sci 2021; 179: 53-69.
[38] Heo MJ, Kim TH, You JS, Blaya D, Sancho-Bru P and Kim SG. Alcohol dysregulates miR-148a in hepatocytes through FoxO1, facilitating pyroptosis via TXNIP overexpression. Gut 2019; 68: 708-720.