SOX6 and PDCD4 enhance cardiomyocyte apoptosis through LPS-induced miR-499 inhibition

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Abstract Sepsis-induced cardiac apoptosis is one of the major pathogenic factors in myocardial dysfunction. As it enhances numerous proinflammatory factors, lipopolysaccharide (LPS) is considered the principal mediator in this pathological process. However, the detailed mechanisms involved are unclear. In this study, we attempted to explore the mechanisms involved in LPS-induced cardiomyocyte apoptosis. We found that LPS stimulation inhibited microRNA (miR)-499 expression and thereby upregulated the expression of SOX6 and PDCD4 in neonatal rat cardiomyocytes. We demonstrate that SOX6 and PDCD4 are target genes of miR-499, and they enhance LPS-induced cardiomyocyte apoptosis by activating the BCL-2 family pathway. The apoptosis process enhanced by overexpression of SOX6 or PDCD4, was rescued by the cardiac-abundant miR-499. Overexpression of miR-499 protected the cardiomyocytes against LPS-induced apoptosis. In brief, our results demonstrate the existence of a miR-499-SOX6/PDCD4-BCL-2 family pathway in cardiomyocytes in response to LPS stimulation.

Keywords SOX6 · PDCD4 · LPS · miR-499 · Cardiomyocyte · Apoptosis

Introduction

Sepsis-induced myocardial functional disorder is one of the main predictors of morbidity and mortality of sepsis [1]; apoptosis is one of the major contributors to the pathophysiology of sepsis [2]. Mediators of sepsis such as lipopolysaccharide (LPS), a gram-negative bacterial cell wall component, triggers apoptosis in cardiac myocytes by promoting the secretion of cytokines such as tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), IL-10, and interferon (IFN)-γ [3].

The SOX (sex-determining region [SRY]-related HMG box) genes were initially identified based on their homology to the HMG box (DNA-binding domain) that is highly similar to that of the SRY protein. The SOX family comprises 20 genes classified into eight groups [4]. SOX6 belongs to the Sox D family, and plays important roles in vertebrate development [5–7]. It also induces apoptosis in esophageal squamous cell carcinoma (ESCC) [8, 9]. Previously, we demonstrated that SOX6 promoted apoptosis at the late stage of P19CL6 cell cardiac differentiation [10]. However, whether Sox6 is involved in LPS-stimulated cardiomyocyte apoptosis is unclear.

MicroRNAs (miRNAs) exert remarkable effects in diverse apoptosis mechanisms involving SOX6. MiR-16 inhibited apoptosis of ESCC cells by downregulating...
apoptosis is unclear. abundant miR-499 in LPS-stimulated cardiomyocyte upregulated LPS-induced cardiomyocyte apoptosis. LPS stimulation PDCD4 cardiomyocyte apoptosis by suppressing miR-499. Currently, whether PDCD4 participates in LPS-induced apoptosis is unknown, as is the possible mechanism involved.

In addition, one miRNA may target several genes; one gene may be regulated by more than one miRNA. MiR-499 regulates apoptosis through multi-gene targeting. Previously, we discovered that PDCD4 (programmed cell death 4) was a target of miR-499 in the regulation of hydrogen peroxide (H₂O₂)-induced apoptosis [21], where PDCD4 is upregulated during apoptosis [22]. Subsequently, a number of reports have indicated that it might participate in some of its physiological and pathophysiological processes. Indeed, it was regulated and functioned differentially in heart development [6, 19, 20]. Whether miR-499 participates in LPS-induced apoptosis is unknown, as is the possible mechanism involved.

In this study, we investigated whether SOX6 and PDCD4, under common control by miR-499, participate in LPS-induced cardiomyocyte apoptosis. LPS stimulation upregulated SOX6 and PDCD4 expression and enhanced cardiomyocyte apoptosis by suppressing miR-499.

**Materials and methods**

**Cell culture and chemical reagents**

Neonatal rat ventricular myocytes were prepared from 1- or 2-day-old Sprague–Dawley rats as previously described [30]. The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 15% fetal bovine serum (FBS) for 36 h, then the culture medium was changed to serum-free medium and the cells were cultured for another 12 h before further experiments. H9c2 cells (ATCC® CRL-1446™, Manassas, VA, USA) and HeLa cells (ATCC® CCL-2™) were maintained in high-glucose DMEM supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 U/mL). LPS (L4391-1MG) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in phosphate-buffered saline (PBS).

**Plasmid constructs and oligonucleotides**

pCMV-SPORT-Pdcd4 (containing the full-length coding sequence of Pdcd4) was a gift from Dr. Iwata Ozaki (Health Administration Center, Department of Internal Medicine, Saga Medical School, Saga University). pGL3-Pdcd4-3’-UTR was kindly provided by Dr. Giridhar Mudduluru (Department of Experimental Surgery Mannheim/Molecular Oncology of Solid Tumors, Deutsches Krebsforschungszentrum and University Heidelberg). pMIR-REPORT-Pdcd4-3’-UTR was constructed by cloning the 3’-untranslated region (3′-UTR) of PDCD4 from pGL3-Pdcd4-3’-UTR into pMIR-REPORT. pcDNA3.1-Sox6 (containing the full-length coding sequence of SOX6) was provided by Dr. Veronique Lefebvre (Case Western Reserve University). pMIR-REPORT-Sox6-3’-UTR and its mutant construct have been described previously [10]. Small interfering RNAs (siRNAs) targeting PDCD4 and SOX6 (Table 1) were commercially synthesized by Sigma-Aldrich and GeneChem (Shanghai, China), respectively. A scrambled 22-nucleotide (nt) miRNA (negative control [NC]), miR-499 Mimic and miR-499 Inhibitor were obtained from RiboBio (Guangzhou, China).

**Luciferase assays**

HeLa cells were plated in 24-well plates at 5 × 10⁴ cells/well 24 h before transfection. PDCD4 and SOX6 luciferase plasmids (400 ng) and 20 ng control Renilla vector were cotransfected with transfection reagent (Lipofectamine® 2000; Invitrogen, Carlsbad, CA, USA). Lysates were collected 48 h after transfection, and luciferase activity was measured in triplicate using a dual luciferase assay (Vigorous, Beijing, China).

| Table 1 | siRNAs used for transfection |
|---------|-----------------------------|
| Gene    | Sequence (5’–3’)             |
| Pdcd4   | Sense: GUCUAAAGGU GGAAAGCG Ud TdT |
|         | Anti-sense: ACCGCUUCCA CCUUAGACd TdT |
| Sox6    | Sense: CACUUGACGUACCAUUCATT |
|         | Anti-sense: UGAAUGGUAUGUGAUGTT |
Western blotting analysis

Total protein extracts were obtained with lysis buffer (150 mM NaCl, 10 mM Tris [pH 7.2], 5 mM EDTA, 0.1 % sodium dodecyl sulfate [SDS], 1 % sodium deoxycholate, 1 % Triton X-100) containing protease inhibitor cocktail (Sigma-Aldrich). Proteins were separated by electrophoresis on 8–15 % SDS–polyacrylamide gels, transferred to nitrocellulose membranes, and incubated with the corresponding primary antibodies. PDCD4 (sc-27123), BAD (sc-8044), BAX (sc-493), BID (sc-6538), and BCL-xL (sc-8392) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); SOX6 (ab64946) antibody was purchased from Abcam (Hong Kong, China); α-actinin (A7811) was from Sigma-Aldrich. The membranes were also probed for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a loading control. The blots were next incubated with peroxidase-conjugated immunoglobulin G secondary antibody and were developed using an enhanced chemiluminescence kit (Millipore, Billerica, MA, USA).

Quantitative real-time PCR

Total RNA was isolated with TRIZol (Invitrogen), and 2 μg total RNA was reverse-transcribed with random primers for complementary DNA (cDNA) synthesis. The cDNA was used for PCR using specific primers. Transcript levels were normalized to 18S rRNA. The primers are listed in Table 2. Each value represents the average of at least three independent experiments.

TUNEL assay and annexin V/PI staining

Cardiomyocyte apoptosis was measured using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (Roche Life Science, Indianapolis, IN, USA) according to the manufacturer’s protocol. Cells cultured on coverslips in 24-well plates were fixed in 4 % paraformaldehyde. The number of TUNEL-positive cells was counted under a fluorescence microscope. For flow cytometry analysis, briefly, cultured cells were harvested by trypsinization and washed with PBS. Cells (1 × 10^6) from each sample were processed for annexin V/propidium iodide (PI) apoptosis detection (Dojindo, Kumamoto, Japan) according to the manufacturer’s instructions.

Statistical analysis

The data are reported as the mean ± standard deviation. Comparisons were analyzed using Student’s t test or ANOVA. p < 0.05 was considered to indicate statistical significance.

Results

MiR-499 levels were downregulated in response to LPS stimulation

Cardiac-abundant miRNAs such as miR-1, miR-133, miR-208, and miR-499 regulate diverse aspects of cardiac function, including cardiomyocyte proliferation, differentiation, contractility, and stress responsiveness. To examine their roles in cardiac cell response to LPS stimulation, we treated cardiomyocytes with 1 μg/mL LPS. Short exposure (6 h) to LPS decreased miR-499 expression, but did not alter expression of the other miRNAs significantly. A known LPS-responsive miRNA, miR-21 [25], was used as the positive control, and showed decreased expression (Fig. 1a). The LPS-induced expression of miR-499 in the cardiomyocytes was concentration- and time-dependent (Fig. 1b, c).

MiR-499 protected cardiomyocytes from LPS-induced apoptosis

To study whether miR-499 participates in LPS-induced apoptosis, we established overexpression and knockdown systems by transfecting cardiomyocytes with miR-499 Mimic (chemically synthesized fragments with the same sequence as miR-499 that enhance endogenous miR-499 function) or with miR-499 Inhibitor (chemically synthesized fragments with reversed complementary sequence to miR-499 that weaken endogenous miR-499 effects), a scrambled 22-nt miRNA was used as the NC (Fig. 2a). Flow cytometry (Fig. 2b, c) and TUNEL (Fig. 2d, e) were employed to verify apoptotic cell numbers. The Mimic-treated cardiomyocytes were less susceptible to 1 μg/mL LPS treated for 6 h, with a lower apoptosis rate than the

Table 2 Primers used for quantitative real-time RT-PCR

| Primers | Sequence (5′–3′) | Product size (bp) |
|---------|-----------------|------------------|
| Pdcd4   | F: TGCCCGTGTT GGCAGTTGTC | 190 |
|         | R: TGGCCCCAACAACCTGTTGTC | |
| Sox6    | F: CCCCCTCTGAACTGGTGGTGGC | 145 |
|         | R: TGGAGCTGGCCCCCTGCCTGAGT | |
| Bad     | F: AAGTCGCCATCCCGGGAATCC | 106 |
|         | R: GCTCACAATCGGTCTAACTTCT | |
| Bax     | F: AGTTGGATGACCGGCTTCCGGG | 156 |
|         | R: GGGGCGTGCTCCAAAAGTTCG | |
| Bid     | F: TCTGAGGTTCGCAGGGTCCTAGT | 95 |
|         | R: CTCTTGGGCGGATACCGCCAG | |
| Bcl-xL  | F: CTGAGGCCTTTACCCGCGGAG | 235 |
|         | R: CAAAGGACATCCCCAGCCTCGT | |
NC-treated cardiomyocytes; while apoptosis was potentiated in Inhibitor-treated cardiomyocytes compared to the NC-treated group. Taken together, these results indicate that the miR-499 level is important for maintaining cardiac cell survival in response to LPS stimulation.

SOX6 and PDCD4 participated in LPS-induced cardiomyocyte apoptosis

To elucidate the molecular mechanisms by which miR-499 regulates apoptosis, we focused on SOX6 and PDCD4, which play important roles in cardiomyocyte differentiation of P19CL6 cells [10] and in protecting adult cardiomyocytes against oxidative stress [21], respectively. SOX6 and PDCD4 expression was first examined to determine whether it is related to LPS-induced cardiomyocyte apoptosis. The results show that SOX6 and PDCD4 expression at both mRNA and protein levels was upregulated in response to 1 μg/mL LPS stimulation (Figs. 3a, b, S1). To explore how SOX6 and PDCD4 are integrated into the cell death program triggered by LPS, the H9c2 cell line was selected for further study. H9c2 is a cardiac myoblast cell line, similar to primary cardiomyocytes [31]. In response to LPS stimulation, SOX6 and PDCD4 overexpression enhanced the rate of apoptosis from 50 % to almost 80 % (Fig. 3c); knockdown of either SOX6 or PDCD4 decreased the rate of apoptosis from approximately 50–25 % (Fig. 3d). However, without LPS stimulation, the overexpression of SOX6 and PDCD4 only slightly increased the apoptosis rate from 6 to 9 % or 13 % respectively (Fig. S2a), while knockdown of either SOX6 or PDCD4 did not affect the apoptosis rate (Fig. S2b), indicating that the effect of SOX6 and PDCD4 is more significant under the circumstance of LPS-induced damage.

Taken together, our observations suggest that SOX6 and PDCD4 promote LPS-mediated apoptosis.

SOX6 and PDCD4 were targets of miR-499 in LPS-induced apoptosis

To investigate whether miR-499 affects the expression of the endogenous target genes, we analyzed the SOX6 and PDCD4 sequences and generated luciferase reporters with the 3'-UTR of SOX6 and PDCD4, and the constructs contained a mutated segment of SOX6 (seed sequence AGUCUUA was mutated to AGUCUCA) and PDCD4 (seed sequence AGUCUUA was mutated to AGUCUGC), respectively (Fig. 4a). As shown in Fig. 4b, c, miR-499 Mimic reduced luciferase activity significantly compared to the NC, whereas no effect was observed with the mutant constructs. This effect was specific because there was no change in luciferase reporter activity when the NC was cotransfected with each reporter construct. Meanwhile, Western blot analysis indicated that miR-499 Mimic attenuated SOX6 and PDCD4 expression, whereas miR-499 Inhibitor elevated it (Fig. 4d). These results suggest that SOX6 and PDCD4 both are direct miR-499 targets.

To investigate whether miR-499 inhibited cardiomyocyte apoptosis by suppressing SOX6 and PDCD4, we performed a rescue experiment in miR-499-treated cells by transfecting pcDNA3.1-Sox6 or pCMV-SPORT-Pdcd4 plasmids. Because pcDNA3.1-Sox6 and pCMV-SPORT-Pdcd4 constructs do not contain 3'-UTR in which the miR-499 binding sites locate, they were not targeted by
Therefore, Western blot showed that SOX6 and PDCD4 protein level was highly upregulated (Fig. 4e).

miR-499 activated the SOX6 and PDCD4 pathways

BCL-2 family members are major regulators of mitochondrial integrity and mitochondria-initiated caspase activation. The BCL-2 family has both anti-apoptotic and pro-apoptotic members, and act as the downstream key factors of many apoptosis mediators [32]. To investigate how SOX6 and PDCD4 regulate LPS-induced cardiomyocyte apoptosis, pcDNA3.1-Sox6 and pCMV-SPORT-Pdcd4 overexpression plasmids as well as their specific siRNAs were used to transfet H9c2 cells. Western blotting indicated that the overexpression plasmids and specific siRNAs successfully enhanced or inhibited SOX6 and PDCD4 expression (Fig. S3). In H9c2 cells transfected with SOX6 or PDCD4 overexpressing constructs, the mRNA level of the pro-apoptotic genes (BAD, BAX, BID) was upregulated, whereas that of the anti-apoptotic gene BCL-XL was downregulated with 1 μg/mL LPS for 6 h (Fig. 5a). But without LPS stimulation, the transfection of SOX6 or PDCD4 did not affect these genes’ expression significantly (Fig. S4). In contrast, the mRNA level of BAD, BAX, and BID was significantly decreased in the SOX6 or PDCD4 knockdown system, whereas that of BCL-XL was increased (Fig. 5b).

In LPS-treated rat cardiomyocytes, miR-499 overexpression inhibited Bad, Bax, and Bid mRNA level, and promoted Bcl-xL level (Fig. 5c); meanwhile, the expression...
of these genes was not affected significantly by miR-499 without LPS treatment (data not shown). In LPS-treated rat cardiomyocytes, the cotransfection of Sox6 or Pdcd4 with miR-499 reversed the miR-499-mediated cardiac protective effects, which included upregulation of Bad, Bax, and Bid mRNA/protein, and downregulating Bcl-xL mRNA/protein.

Fig. 3 SOX6 and PDCD4 are involved in LPS-induced cardiac cell apoptosis. a SOX6 and PDCD4 mRNA level in cardiomyocytes exposed to LPS. Cardiomyocytes not treated with LPS (0 μg/mL) were used as the negative control. b SOX6 and PDCD4 protein level in LPS-treated cardiomyocytes. c, d Quantitative flow cytometry results for annexin V/PI-stained cells transfected with SOX6 or PDCD4 plasmid (c) or with SOX6 siRNA or PDCD4 siRNA (d), respectively, and then exposed to 1 μg/mL LPS for 6 h. The vector plasmid pcDNA3 (Empty) or scrambled siRNA were used as the negative control. *p < 0.05, **p < 0.01 compared with negative control. Data represent the results of three independent experiments.

Fig. 4 SOX6 and PDCD4 are targets of miR-499. a Schematic illustration indicates the seed sequences on the 3′-UTR of SOX6 or PDCD4, which are potential target genes of miR-499. The mutant binding sites are underlined. b, c Luciferase analysis of the effect of miR-499 on its potential targets. Luciferase activity was measured in HeLa cells cotransfected with miR-499 and SOX6 3′-UTR luciferase reporter (luc) (wild type [WT]) or SOX6 3′-UTR luc (mutant [Mut]) (b), PDCD4 3′-UTR luc (WT) or PDCD4 3′-UTR luc (Mut) (c). d SOX6 and PDCD4 protein level in cardiomyocytes treated with miR-499 NC, Mimic, or Inhibitor. GAPDH was used as the internal control. e Expression of SOX6 (left) and PDCD4 (right) in H9c2 cells cotransfected with miR-499 Mimic in combination with SOX6 or PDCD4 plasmid, respectively. *p < 0.05, **p < 0.01 compared with negative control. Data represent the results of three independent experiments.
protein (Fig. 5d–f), indicating the existence of an miR-499-Sox6/Pdcd4-apoptosis pathway.

Discussion

Cardiomyocyte apoptosis is one of the major pathogenic factors in heart diseases, including septic cardiomyopathy. Bacterial endotoxins, such as LPS, are considered the principal cause of myocardial dysfunction. However, the mechanisms involved are unclear. In this study, we attempted to explore the mechanisms involved in LPS-induced cardiomyocyte apoptosis. We discovered that LPS stimulation reduced miR-499 expression, thereby relieving the inhibitory effect on its target genes Sox6 and Pdcd4, which then activated the BCL-2 family pathway to participate in LPS-induced apoptosis.

SOx6 is a key regulator in cell differentiation and organ development. It also plays important roles in proliferation and apoptosis during stem cell differentiation or cancer progression. In hepatocellular carcinoma, SOx6 activates p21/WAF1/CIP1 (p21) expression in a p53-dependent manner, and miR-155 targeting of SOx6 facilitated cell proliferation [11]. In pancreatic β-cells, SOX6 inhibited cyclin D1 promoter activity and negatively regulated cell proliferation by interacting with histone deacetylase 1 (HDAC1) and β-catenin [33]. SOX6 suppression also caused retinoic acid-dependent apoptosis and blocked neuronal differentiation in the early stages of P19 cell neuronal differentiation [34]. However, the involvement of
SOX6 in LPS-induced cardiomyocyte apoptosis has not been reported. In our previous research, we found that SOX6, as a repressor of cyclin D1, arrested cardiomyocyte proliferation and facilitated cell cycle exit [10]. The present study is the first demonstration, to our knowledge, that SOX6 expression is elevated in LPS-treated cardiomyocytes in a time- and dose-dependent manner, and the upregulated SOX6 enhances LPS-induced apoptosis.

We also found that PDCD4 was upregulated in LPS-treated cardiomyocytes. PDCD4 is a tumor repressor, suppressing tumor growth through different mechanisms [35]. PDCD4 also plays an important role in various inflammatory diseases. PDCD4-deficient cells were significantly less sensitive to apoptosis, and PDCD4 overexpression in β-TC-6 cells increased their susceptibility to TNF-α plus IFN-γ-induced apoptosis [28]. PDCD4 promoted activation of the transcription factor nuclear factor-kB (NF-kB) and suppressed IL-10; Pdc4-deficient mice were less susceptible to LPS-induced death, suggesting PDCD4 is a proinflammatory factor [25]. However, other reports showed that PDCD4 deficiency increased TNF-α protein expression in LPS-treated RAW264.7 macrophages, indicating that PDCD4 is an anti-inflammatory factor [36, 37]. Therefore, the exact role of PDCD4 in inflammatory diseases remains to be investigated. It should be noted that the effect of LPS stimulation on PDCD4 expression differs between reports. In primary bone marrow-derived macrophages and RAW264.7 macrophages, PDCD4 was upregulated at 1 h after LPS treatment and gradually decreased at 6 h [24]. Another report showed that PDCD4 remained at a high level at 8 h after LPS stimulation in human peripheral blood mononuclear cells [25]. It is easy to understand that dynamic feedback is involved in the response to various stimuli; any difference could lead to different feedback. Nevertheless, we found that PDCD4 was upregulated in LPS-stimulated cardiomyocytes.

To understand how SOX6 and PDCD4 expression is regulated in LPS-treated cardiomyocytes, we detected the expression of a series of miRNAs. MiR-499 and miR-21 were downregulated in LPS-induced cells in a dose- and time-dependent manner, while there was no significant change to miR-1, miR-133, and miR-208 expression. A growing amount of evidence demonstrates that miRNAs can regulate apoptosis [38]. Thirteen miRNAs, including miR-21, were upregulated in early-stage dilated cardiomyopathy, whereas 11 miRNAs including miR-499 were downregulated [39]. MiR-499 was also downregulated in cardiomyocytes exposed to anoxia [40]. In our previous work, we showed that miR-499 and miR-21 were upregulated in H2O2-induced cardiomyocyte apoptosis [21]. Hence, miRNA expression is correlated with various diseases, stimuli, cells, and detection time [41]. Several reports demonstrated that transient transfection of miR-1 and miR-499 reduced proliferation and enhanced differentiation into cardiomyocytes in human cardiac progenitor cells and embryonic stem cells [6]. In a transgenic mouse model, elevated miR-499 levels affected Egr1 and Fos, the immediate early genes in the response to cardiac stress [31]. Additionally, in rat myocardial infarction area induced by anoxia and ischemia, miR-499 inhibited cardiomyocyte apoptosis through regulation of mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1 [40].

In the present study, LPS stimulation suppressed miR-499 expression, which led to elevation of the cardiomyocyte apoptosis rate. Our experiments also showed that the BCL-2 family is involved in miR-499-SOX6-PDCD4 apoptotic regulation. MiR-499 inhibited the expression of pro-apoptotic genes and upregulated expression of the anti-apoptotic gene BCL-XL. SOX6 and PDCD4 reversed these effects, suggesting that miR-499 regulates BAD, BAX, BID, and BCL-XL expression by inhibiting SOX6 and PDCD4, thereby playing a key role in LPS-induced cardiomyocyte apoptosis. To investigate how LPS downregulates miR-499, sequence analysis of the promoter region of MYH7B, the host gene of miR-499, was performed. The result showed that it contains one NF-kB binding site, which is conserved among rats, mice, and humans. NF-kB is activated by LPS-induced TNF-α [2]. However, when we transfected the luciferase reporter construct containing a 1000-bp region of the MYH7B regulatory fragment upstream of the translational start site into H9c2 cells that were then treated with LPS, no significant change in luciferase activity was observed after LPS treatment (data not shown). It should be noticed that we used H9c2 cells for the most experiments to demonstrate SOX6 and PDCD4 as the targets of miR-499. Although this cell line is different from primary neonatal myocytes, it is commonly used as a cardiomyocyte model for mechanism investigation [42, 43]. However, further investigation is required to clarify the upstream regulation mechanism of miR-499. In summary, LPS stimulation relieved the inhibitory effect of miR-499 on its target genes SOX6 and PDCD4, which enhanced LPS-induced cardiomyocyte apoptosis through the BCL-2 family members. Overexpression of miR-499 protected cardiomyocytes against LPS-induced apoptosis.

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Compliance with ethical standards
Conflict of interests The authors declare no conflict of interest.
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