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ORIGINAL ARTICLE

CANCER INNOVATION

Resveratrol activation of SIRT1/MFN2 can improve mitochondria function, alleviating doxorubicin-induced myocardial injury

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Abstract

Background: Doxorubicin is a widely used cytotoxic chemotherapy agent for treating different malignancies. However, its use is associated with dose-dependent cardiotoxicity, causing irreversible myocardial damage and significantly reducing the patient's quality of life and survival. In this study, an animal model of doxorubicin-induced cardiomyopathy was used to investigate the pathogenesis of doxorubicin-induced myocardial injury. This study also investigated a possible treatment strategy for alleviating myocardial injury through resveratrol therapy in vitro.

Methods: Adult male C57BL/6J mice were randomly divided into a control group and a doxorubicin group. Body weight, echocardiography, surface electrocardiogram, and myocardial histomorphology were measured. The mechanisms of doxorubicin cardiotoxicity in H9c2 cell lines were explored by comparing three groups (phosphate-buffered saline, doxorubicin, and doxorubicin with resveratrol).

Results: Compared to the control group, the doxorubicin group showed a lower body weight and higher systolic arterial pressure, associated with reduced left ventricular ejection fraction and left ventricular fractional shortening, prolonged PR interval, and QT interval. These abnormalities were associated with vacuolation and increased disorder in the mitochondria of

Abbreviations: Bax, B-cell lymphoma-2-associated X; CV, conduction velocity; DBP, diastolic blood pressure; ETC, electron transport chain; HE, hematoxylin-eosin; IVSd, interventricular septal thickness end-diastolic; IVSs, interventricular septal thickness end-systolic; LVEF, left ventricular ejection fractions; LVFS, left ventricular fractional shortening; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVM, left ventricular mass; LVPWd, left ventricular posterior wall thickness end-diastolic; LVPWs, left ventricular posterior wall thickness end-systolic; MBP, mean blood pressure; MFN2, Mitofusin2; MMP, mitochondrial membrane potential; MnSOD, manganese superoxide dismutase; NAD, adenine dinucleotide; PBS, buffered saline group; ROS, reactive oxygen species; SBP, systolic blood pressure; SIRT1, Sirtuin1; α-SMA, α-smooth muscle actin.

Qingling Zhang and Yunpeng Zhang contributed equally to this study and shared the first authorship.

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cardiomyocytes, increased protein expression levels of α-smooth muscle actin and caspase 3, and reduced protein expression levels of Mitofusin2 (MFN2) and Sirtuin1 (SIRT1). Compared to the doxorubicin group, doxorubicin + resveratrol treatment reduced caspase 3 and manganese superoxide dismutase, and increased MFN2 and SIRT1 expression levels.

Conclusion: Doxorubicin toxicity leads to abnormal mitochondrial morphology and dysfunction in cardiomyocytes and induces apoptosis by interfering with mitochondrial fusion. Resveratrol ameliorates doxorubicin-induced cardiotoxicity by activating SIRT1/MFN2 to improve mitochondria function.

KEYWORDS

cardio-oncology, doxorubicin-induced cardiomyopathy, mitochondria function, resveratrol, SIRT1 agonists

1 | INTRODUCTION

Doxorubicin, an anthracycline antibiotic extracted from *Streptomyces* [1], is effective against solid malignancies such as breast cancer, as well as soft tissue sarcoma, Hodgkin's lymphoma, and leukemia. Although doxorubicin has a broad antitumor spectrum, its cardiotoxicity where it causes adverse cardiac remodeling and heart failure limits its clinical application. Clinical studies have shown that doxorubicin could induce heart failure at cumulative body doses exceeding 400–700 and 300 mg/m^2 in adult and pediatric cancer survivors, respectively [2–4]. The risk of death from heart disease 10 years later in breast cancer patients exceeds that of the tumor itself [5]; however, the mechanism of doxorubicin cardiotoxicity has not been elucidated fully. Current research focuses on oxidative stress, mitochondrial dysfunction, myocardial fibrosis, endoplasmic reticulum stress, DNA damage and apoptosis, dysregulation of autophagy, and ferroptosis [6–12].

Mitochondria are abundant in cardiomyocytes [13, 14], and mitochondria-rich cardiomyocytes are susceptible to doxorubicin toxicity. Mitochondrial quality control is mediated by a homeostatic balance between mitochondrial biogenesis and degradation, mainly regulated by dynamic processes such as mitochondrial fission and fusion, mitochondrial cristae remodeling, mitochondrial biosynthesis, Ca^{2+} homeostasis, and mitophagy [15]. In various animal models, mitochondrial damage produces a large number of oxidative substances that lead to myocardial apoptosis, ultimately leading to remodeling of the atria [16–20] and/or ventricles [18, 21, 22], and decreased cardiac function. MFN2 is a mitochondrial fusion protein mainly involved in the fusion of mitochondria. Structural analysis of MFN2 revealed a Ras binding site at its N-terminus [23, 24], suggesting that it may have functions other than mediating mitochondrial fusion. Studies have shown that MFN2 plays an important role in regulating cellular energy metabolism, proliferation and apoptosis, and signal transduction [24–27]. Mitochondrial respiration was significantly impaired in MFN2-deficient fibroblasts with reduced mitochondrial membrane potential and glucose oxidative capacity [28]. MFN2 gene knockout can lead to the depletion of mtDNA [29]. Karbowski et al. [30] found an MFN2 and B-cell lymphoma-2-associated X (Bax) co-localization phenomenon on mitochondria in discrete foci during apoptosis, suggesting that MFN2 is involved in the regulation of apoptosis.

SIRT1 is a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase that participates in pathophysiological processes such as cardiomyocyte survival, metabolic homeostasis, and aging through deacetylation-related factors [31–35], and in myocardial protection by enhancing the ability of cells to resist oxidative stress, reducing cell apoptosis/pyroptosis and inflammatory response, regulating energy metabolism, and coordinating cardiac diastolic and systolic function [36–42]. A previous study shows that the expression level of SIRT1 in cardiomyocytes is downregulated in advanced heart failure [43]. Activation of SIRT1 in cardiomyocytes can improve mitochondrial function, activate oxidative stress resistance pathways, and attenuate doxorubicin cardiotoxicity [44–46]. Upregulation of MFN2 by SIRT1 improves prognosis in ischemic cardiomyopathy [47]. However, the mechanism of SIRT1 and MFN2 expression in doxorubicin-induced cardiac pathology is not fully understood. We established an animal model of doxorubicin-induced cardiomyopathy and a doxorubicin-injured cardiomyocyte model to verify our hypothesis: resveratrol attenuates

doxorubicin cardiac/cardiomyocyte cytotoxicity by activating SIRT1 to promote MFN2 expression in cardiomyocytes. Our work adds a rationale for further explaining doxorubicin cardiotoxicity and provides a new option for preventing or delaying doxorubicin-induced cardiomyopathy.

2 | METHODS

The methods are provided in detail in the Supporting Information Materials and Methods.

3 | RESULTS

3.1 | The effects of doxorubicin on body metrics and physiological parameters

The effects of doxorubicin on body metrics were examined. A schematic diagram of animal intervention is shown in Figure 1a. During the course of the experimental study of 10 days, the weight of the control group increased by an average of (6.12 ± 2.59) %, while that of the doxorubicin group decreased by an average of (9.42 ± 3.13) %, $p < 0.05$, (Figure 1b). Regarding hemodynamic measurements, there was no significant difference between the control and doxorubicin groups in terms of systolic, diastolic, and mean blood pressure or heart rate (Figure 1c,d) at the baseline. Blood pressure ($[112.83 \pm 5.9]$ vs. $[120.67 \pm 7.07]$ mmHg, $p = 0.03$) and heart rate ($[593.55 \pm 47.4]$ vs. $[665.33 \pm 39.6]$ bpm, $p = 0.006$) were significantly higher in doxorubicin mice compared to their baseline data (Figure 1e,f).

3.2 | Doxorubicin led to structural and electrical remodeling in the heart

To study the effects of doxorubicin on cardiac function, echocardiography was performed (Figure 2a). Compared to the control group, the LVEF ($[50.77 \pm 7.37]$ % vs. $[39.16 \pm 8.14]$ %, $p = 0.010$), and LVFS ($[25.51 \pm 4.48]$ % vs. $[18.95 \pm 4.58]$ %, $p = 0.012$) of the doxorubicin group were significantly lower than those of the control group, while LVIDs (left ventricular internal diameter in systole) ($[2.84 \pm 0.38]$ vs. $[3.42 \pm 0.19]$ mm, $p = 0.003$) and LVIDd (left ventricular internal diameter in diastole) ($[3.81 \pm 0.38]$ vs. $[4.23 \pm 0.27]$ mm, $p = 0.02$) were significantly increased compared with the control group (Figure 2c–f). The remaining echocardiographic parameters were not statistically different (Supporting Information: Table 1). The surface electrocardiogram (Figure 2b)

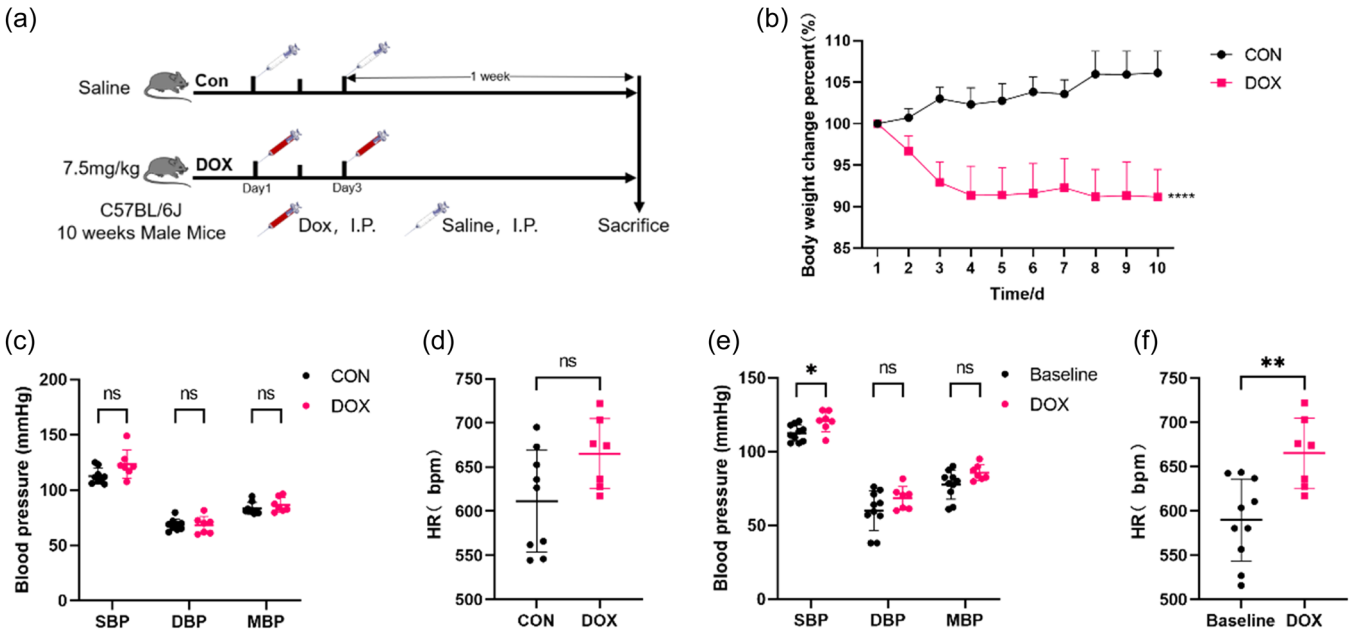

FIGURE 1 The effects of doxorubicin on the general vital sign resulted in a loss of weight and an increase in aortic blood pressure. (a) Adult male C57BL/6J mice (10 weeks old) were randomly divided into a control group (CON) and a doxorubicin group (DOX) which was injected with doxorubicin (7.5 mg/kg, twice every other day, intraperitoneally). (b) Weight change was calculated as a percentage change in weight compared with the baseline. (c, d) Blood pressure and heart rate in the two groups; (e, f) blood pressure and heart rate were observed in the doxorubicin group compared with the baseline ($n = 7$–10). Data are mean ± SD; *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. DBP, diastolic blood pressure; HR, heart rate; MBP, mean blood pressure; SBP, systolic blood pressure.

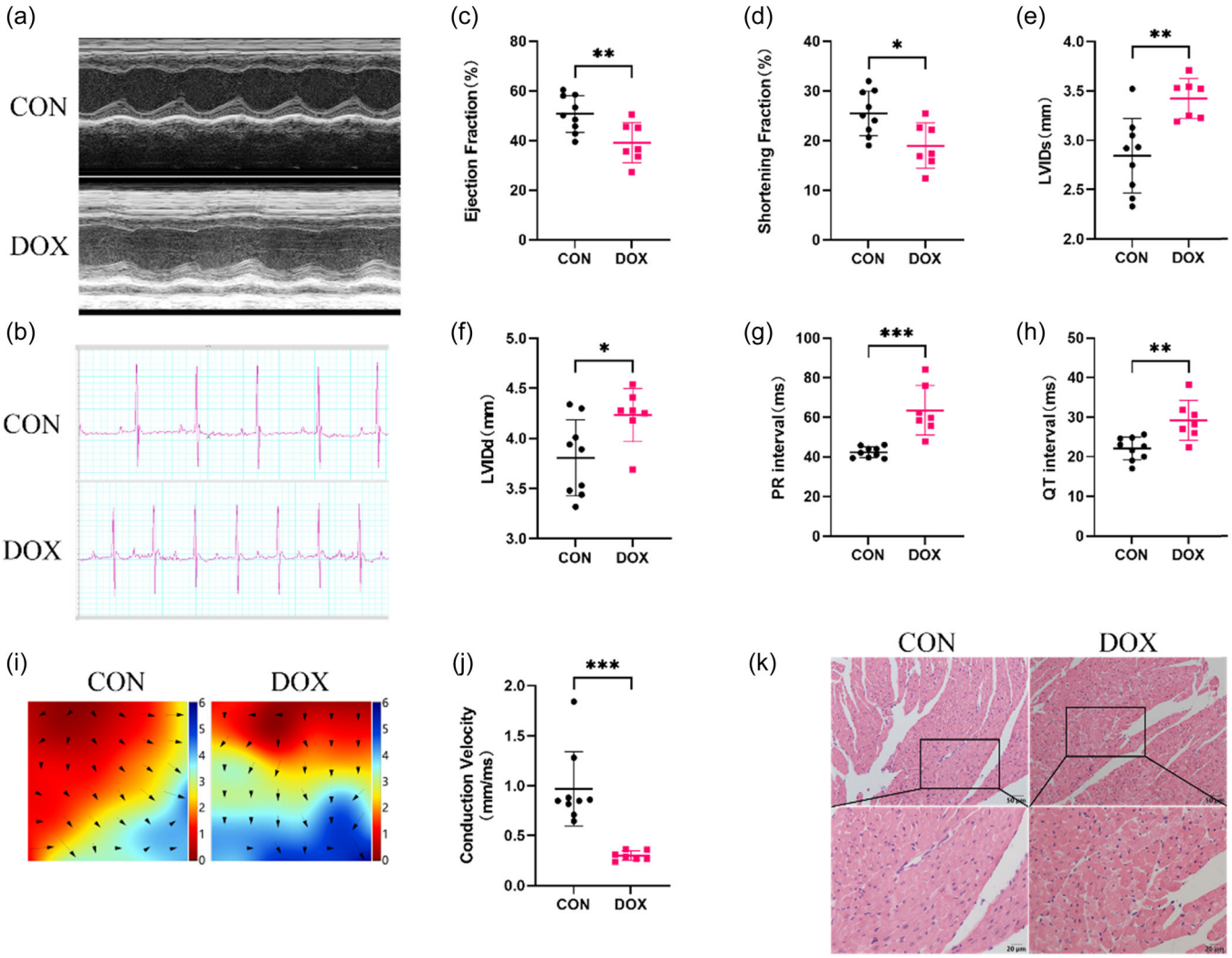

FIGURE 2 Doxorubicin causes structural and electrical remodeling of the heart. (a) Representative figures for M-mode of echocardiographic images in the two groups; (b) typical body surface ECGs in the two groups; (c–f) analysis results of left ventricular echocardiographic parameters in each group; (g–h) Results of ECG parameters between groups. (i, j) The representative figures for mapping and the results for conduction velocity. (k) Representative images of HE staining, scale bar, 20 and 50 μm. Data are mean ± SD; $n = 7–9$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CON, control group; DOX, doxorubicin group; LVIDd, left ventricular internal dimension end-diastolic; LVIDs, left ventricular internal dimensions end-systolic.

showed that compared with the control group, the PR interval ([42.31 ± 2.74] vs. [63.46 ± 12.48] ms, $p < 0.001$) and QT interval ([22.02 ± 2.90] vs. [29.17 ± 5.03] ms, $p = 0.003$) of the doxorubicin group were significantly longer than those of the control group (Figure 2g,h). Epicardial electrical mapping (Figure 2i) showed lower cardiac conduction velocity in the doxorubicin group ([0.97 ± 0.37] vs. [0.30 ± 0.05] mm/ms, $p < 0.001$) (Figure 2j), with no statistically significant difference in absolute inhomogeneity ($p = 0.429$), or conduction inhomogeneity index ($p = 0.9275$) (Supporting Information: Figure 1). Morphological changes in cardiomyocytes were observed by H&E staining. The results showed that the myocardial structure of ventricular tissue in the doxorubicin group was destroyed, with the myocardial cells undergoing extensive vacuolization and disorderly arrangement, compared to the control group that showed normal morphology (Figure 2k).

3.3 | Doxorubicin impaired cardiomyocyte mitochondrial function and disrupted mitochondrial homeostasis

The mechanism of doxorubicin-induced cardiotoxicity is complex and irreversible, including increased in oxidative stress, inhibition of topoisomerase 2β, interference with mitochondrial function, and apoptosis

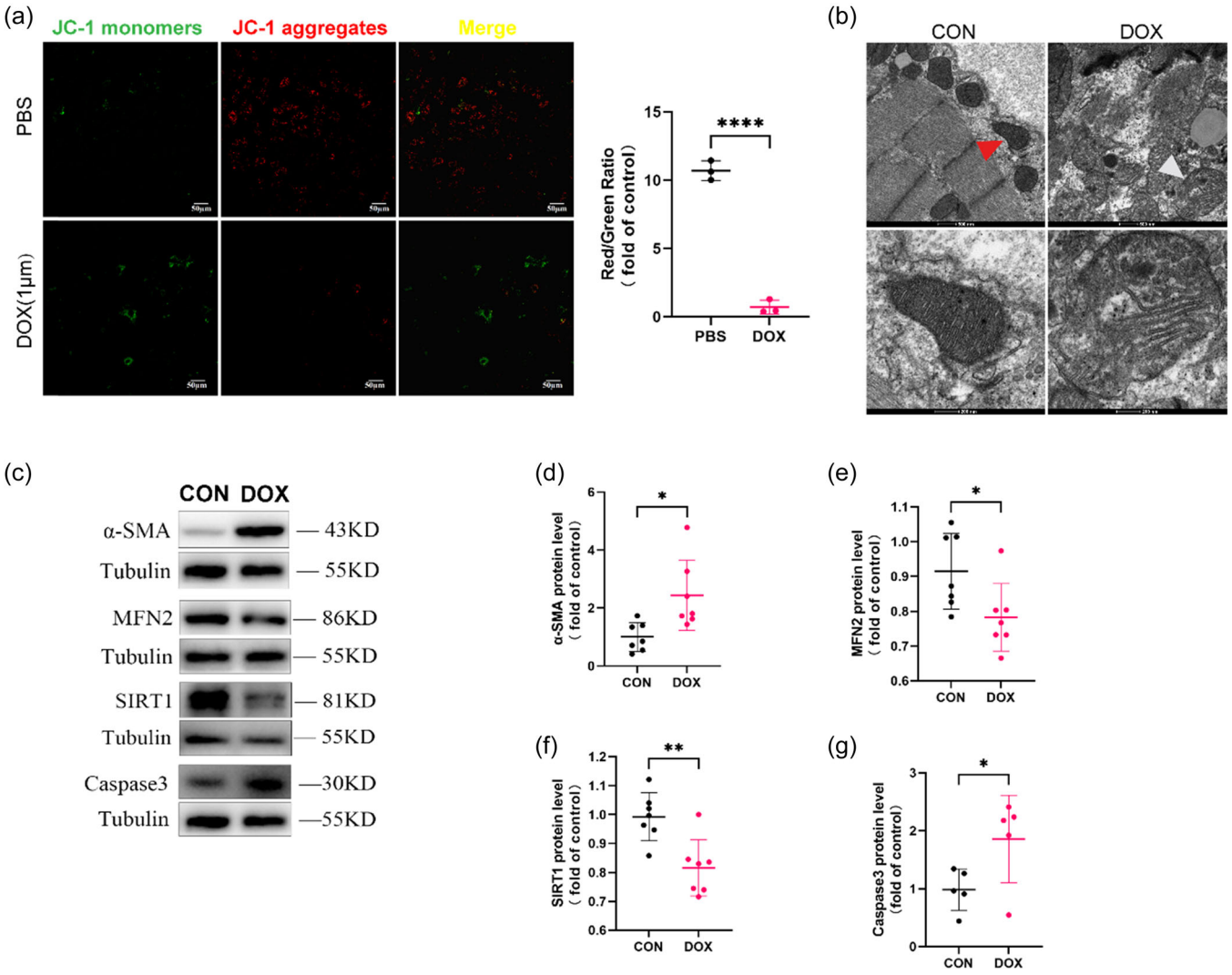

FIGURE 3 Doxorubicin impaired cardiomyocyte mitochondrial function and disrupted mitochondrial homeostasis. (a) Representative confocal microscopy images of mitochondrial membrane potential fluorescence staining and its statistics; the ratio of red/green fluorescence reflects the membrane potential changes. Scale bar, 50 μm. $n = 3$ independent experiments. (b) Transmission electron microscopy images of mitochondria in mice ventricular cardiomyocyte. Red arrows show normal mitochondrial morphology; white arrows show damaged mitochondrial morphology. $N = 3$. Scale bar, 200 and 500 nm; (c) Expression of α-SMA, MFN2, SIRT1, and caspases3 protein in mice ventricular tissues among two groups ($n = 5$–7). (d–g) Quantification of α-SMA, MFN2, SIRT1, and caspases3 in (c). Data are mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. α-SMA, α-smooth muscle actin; CON, control group; DOX, doxorubicin group; MFN2, Mitofusin2; PBS, phosphate-buffered saline, the control group in H9c2 cells; SIRT1, Sirtuin1.

[48, 49]. We detected mitochondrial membrane potential (MMP) changes in doxorubicin-treated H9c2 cells. JC-1 results showed that MMP levels in ventricular myocytes were significantly reduced after 1 μM doxorubicin treatment compared with the PBS group (Figure 3a); mitochondrial morphology of mouse ventricular cardiomyocytes was examined using transmission electron microscopy. Mitochondrial membrane rupture and blebbing were observed, which was associated with increased membrane density, decreased inner membrane cristae, and incomplete inner and outer membrane structures (Figure 3b). The protein expression levels of α-SMA, MFN2, SIRT1, and Caspase 3 in ventricular tissue were demonstrated using Western blot (Figure 3c). The results showed that compared with the control group, the expressions of α-SMA and Caspase 3 in the doxorubicin group were significantly increased, and the expressions of SIRT1 and MFN2 were downregulated in the doxorubicin group (Figure 3d–g).

The above results suggested that doxorubicin interferes with mitochondrial division, impoverishes mitochondrial functions, and causes apoptosis and fibroids in the cardiomyocytes that eventually cause cardiotoxicity.

3.4 | Doxorubicin increases oxidative stress in cardiomyocytes which could be reversed by resveratrol

Doxorubicin increases oxidative stress in cardiomyocytes, causing damage to mitochondria [9, 11]. To further investigate the level of intracellular oxidative stress after doxorubicin treatment, the DCFH-DA fluorescent probe was used to detect the level of intracellular reactive oxygen species (ROS), with ROS levels indicated by the green fluorescence in Figure 4a. When the doxorubicin concentration reached 2 μM, the level of ROS in ventricular myocytes was significantly increased (Figure 4b). Previous studies have shown that resveratrol has antioxidative stress effects [36]. We applied resveratrol to H9c2 cells as a pretreatment. The results showed that ROS levels were significantly decreased in the resveratrol-treated group compared with the control group (Figure 4c,d). At the same time, we measured the expression level of the oxidative stress indicator, MnSOD protein, in cardiomyocytes (Figure 4e). The results showed that the expression of MnSOD was increased in the doxorubicin group, but the level of MnSOD in cardiomyocytes was significantly downregulated in the resveratrol-treated group (Figure 4f), indicating that resveratrol reduces oxidative stress caused by doxorubicin.

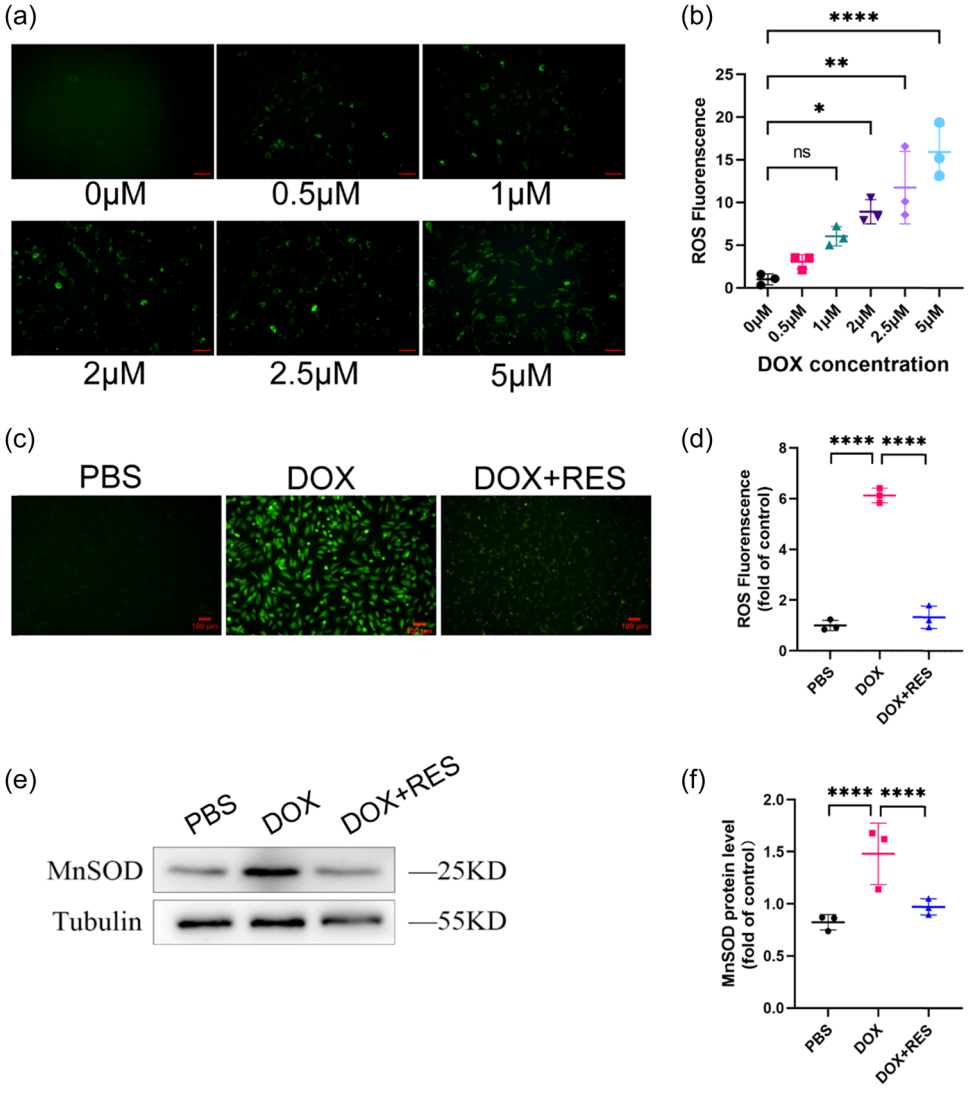

FIGURE 4 Doxorubicin increased oxidative stress in cardiomyocytes which could be reversed by resveratrol. (a) Representative DCFH-DA fluorescent images in H9c2 cells treated with different concentrations of doxorubicin. Scale bar, 200 μm. (b) DCFH-DA fluorescence intensity quantification. (c) Representative DCFH-DA fluorescent images in each group. Scale bar, 100 μm. Quantification of DCFH-DA in (d). (e) Expression of Mn-SOD protein among the three groups. $n = 3$, for independent experiments. (f) Quantification of Mn-SOD protein expression in (e). *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. Data are mean ± SD. DOX, doxorubicin group; DOX + RES, doxorubicin + resveratrol group; MnSOD, manganese superoxide dismutase; PBS, phosphate-buffered saline, the control group in H9c2 cells.

3.5 | Resveratrol promotes the expression of SIRT1 and MFN2 in cardiomyocytes, which improves mitochondrial homeostasis and reverses doxorubicin damage

In our animal model, we found that the protein expression of SIRT1 and MFN2 was significantly downregulated in the doxorubicin group. As a classic SIRT1 protein agonist, we hypothesized that resveratrol could ameliorate doxorubicin-induced myocardial injury.

Compared with the PBS group, the MMP levels of H9c2 cells were significantly decreased after 1 μM doxorubicin treatment, but this mitochondrial membrane potential damage improved after resveratrol administration (Figure 5a,b). We further explored the levels of mitochondria-related proteins. The results show that compared with the doxorubicin group, the expression of SIRT1 protein was significantly upregulated in the doxorubicin + resveratrol group after the administration of resveratrol, while the expression level of MFN2 was restored (Figure 5c,d).

Mitochondria play an important role in apoptosis [50]. The proapoptotic protein Caspase 3 is a key apoptosis-enforcing proteolytic enzyme downstream of the apoptotic cascade [51]. We found that its expression

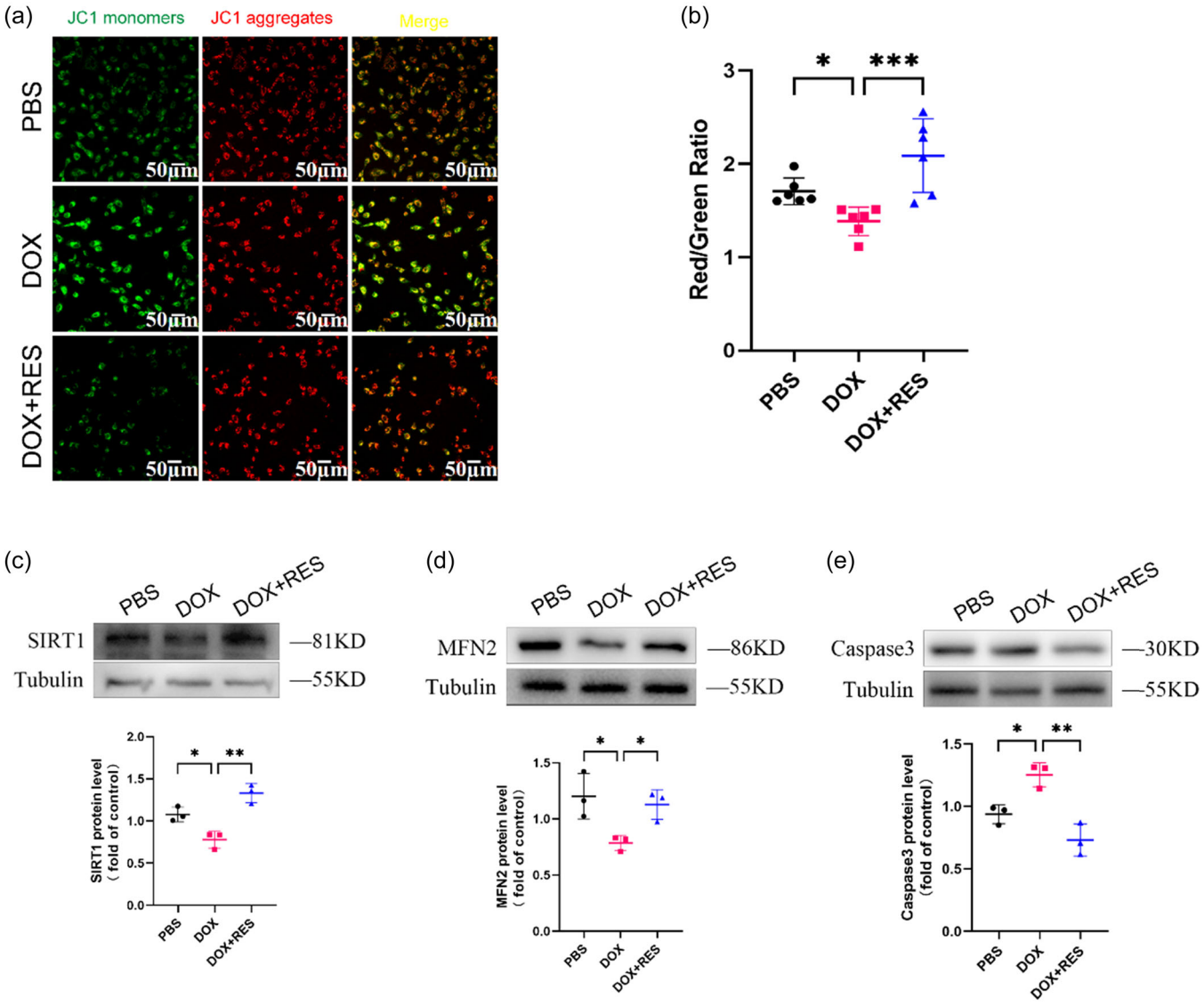

FIGURE 5 Resveratrol promoted the expression of SIRT1 and MFN2 in cardiomyocytes, which improved mitochondrial homeostasis and reversed doxorubicin damage. (a) Representative confocal microscopy images of mitochondrial membrane potential fluorescence staining and its statistics; the ratio of red/green fluorescence reflects the membrane potential changes. Scale bar, 50 μm. $n = 3$. (b) Quantification of DCFH-DA in (a). (c–e) Representative western blot results of expression of SIRT1, MFN2, and Caspases3 protein among the three groups. Data are mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. DOX, doxorubicin group; DOX + RES, doxorubicin + resveratrol group; MFN2, Mitofusin2; PBS, control group in H9c2 cells; SIRT1, Sirtuin1.

was significantly upregulated in doxorubicin-treated animal models. To further explore whether resveratrol improved mitochondrial apoptosis, we conducted further experiments at the cellular level. The results showed that, compared with the doxorubicin group, the doxorubicin + resveratrol group showed decreased Caspase 3 levels and a significant decrease in the level of apoptosis (Figure 5e) ($p < 0.05$).

4 | DISCUSSION

Anticancer drugs have side effect profiles such as increased toxicity to the cardiovascular system [52–60]. For doxorubicin-related cardiotoxicity, interventions that reduce oxidation stress have failed to reduce cardiac damage in patients [12]. Therefore, potential therapeutic mechanisms of existing drugs are needed to identify better pharmacotherapies and interventions. Our study found that the SIRT1 agonist, resveratrol, plays an important role in the regulation of myocardial oxidative stress and mitochondrial homeostasis during the progression of doxorubicin-induced cardiotoxicity. Specifically, (1) doxorubicin-induced myocardial mitochondrial injury manifests as mitochondrial dysfunction and downregulation of MFN2 expression; (2) in doxorubicin-induced myocardial injury, the level of cellular oxidative stress was increased, the expression of SIRT1 was downregulated, and the expression of apoptosis marker Caspase 3 was upregulated, indicating higher rates of apoptosis; (3) The SIRT1 agonist, resveratrol, attenuates the cardiotoxicity of doxorubicin by upregulating MFN2 expression, reducing the level of oxidative stress in cardiomyocytes.

The fusion and fission of mitochondria are coordinated and are highly conserved processes mediated by the precise regulation of multiple proteins. The two maintain a dynamic balance to maintain the normal shape, function, and distribution of mitochondria. MFN2 is an essential member of the mitochondrial fusion protein family. Previous studies have found that the mitochondrial fusion efficiency of MFN2-deficient cells is significantly reduced, 69% of mitochondria are not fused, only 1% of mitochondria are fully fused, and mitochondria are fragmented. If the MFN2 gene is re-expressed, mitochondria will revert to a long tubular shape [61]. The cell's ability to oxidize glucose is reduced, and mitochondrial respiratory function is significantly impaired [28]. Therefore, the ability to maintain mitochondrial homeostasis plays an important role in maintaining the normal function of cardiomyocytes. Mitochondrial morphology, dynamics, and mitochondrial energy metabolism become disordered in doxorubicin-induced cardiomyopathy [6]. Ding et al. found that upregulation of MFN2 expression enhanced mitochondrial oxidative metabolism by mediating mitochondrial fusion, reducing cell damage, and suppressing doxorubicin cardiotoxicity [62]. Doroshow and Davies found that doxorubicin causes cardiac mitochondria to produce superoxide anions and ROS, which in turn lead to disturbances in the mitochondrial electron transport chain, further damaging mitochondria to produce more oxidative species [63, 64]. In the animal model, we found that ROS production was increased, mitochondrial membrane potential was depolarized, and the inner and outer membrane structure was incomplete under an electron microscope after doxorubicin administration. These mitochondrial changes may be related to the downregulation of the mitochondrial fusion protein (MFN2). Previous studies, and our experimental results, also confirm that doxorubicin reduces the expression level of MFN2 in cardiomyocytes. This results in mitochondrial dysfunction, resulting in decreased cardiomyocyte viability. However, some researchers have hypothesized that overexpression of MFN2 alone can induce a decrease in the phosphorylation level of protein kinase B (Akt), triggering apoptosis by inhibiting the Ras-induced PI3K-Akt pathway which promotes cell survival [65], but some studies suggest that the effect of MFN2 on the Ras signaling pathway is independent of the classical profusion effect [65]. Although the above conclusions differ from our results, we believe that the upregulation of MFN2 promotes apoptosis through a nonmitochondrial fusion pathway, which is different from the mitochondrial fusion mechanism in which doxorubicin promotes cardiomyocyte apoptosis through the downregulation of MFN2. Therefore, the complete molecular role of MFN2 still needs to be elucidated.

SIRT1 regulates processes such as apoptosis and muscle differentiation by catalyzing the deacetylation of key proteins and plays an important role in oxidative stress and DNA damage repair [33]. Previous studies have shown that SIRT1 could regulate mitochondrial homeostasis to enhance mitochondrial function or regulate MFN2 expression through direct deacetylation [47, 66–68]. In this study, animal experiments found that doxorubicin stimulation reduced the expression of SIRT1; this result is consistent with previous studies in which doxorubicin reduced the expression of SIRT1 in H9c2 and neonatal rat cardiomyocytes [69]. Therefore, activation of SIRT1 may improve doxorubicin cardiotoxicity. In our study, SIRT1 protein expression was restored, and the level of oxidative stress in cardiomyocytes and the protein level of the apoptosis protein caspase-3 were improved after the use of the SIRT1 agonist resveratrol. Therefore, this suggests that resveratrol may play a

protective role in the development of doxorubicin-related cardiotoxicity through a mechanism that restores mitochondrial function and reduces apoptosis.

Several limitations of our study should be acknowledged. First, although the present findings suggest that mitochondrial dysfunction and apoptosis are involved in doxorubicin-related cardiotoxicity in cellular and animal models, further studies are needed to determine the corresponding roles in humans. Second, resveratrol has a variety of pharmacological effects, and SIRT1 is only one of its targets in vitro and in vivo. Therefore, whether resveratrol also participates in doxorubicin-related cardiotoxicity through other targets needs to be further explored. Finally, we focused solely on the role of the SIRT1 agonist resveratrol in improving mitochondrial function. Further studies are needed to explore the SIRT1 pathway, other mitochondrial functional proteins, and their role in doxorubicin-induced cardiotoxicity.

5 | CONCLUSION

In conclusion, our study shows that doxorubicin significantly decreases mouse survival rate, enhances oxidative stress, decreases mitochondrial membrane potential, downregulates the expression of mitochondrial fusion protein MFN2, and upregulates Mn-SOD and Caspase 3. The SIRT1 agonist resveratrol inhibits cardiomyocyte apoptosis by upregulating MFN2, preventing oxidative stress injury, and thereby ameliorating doxorubicin-induced cardiotoxicity. Thus, the SIRT1/MFN2 axis may be an important new target for the treatment of doxorubicin-induced cardiomyopathy. Meanwhile, resveratrol could be a promising treatment candidate for clinical translation as a novel cardiomyocyte protector to mitigate doxorubicin-induced cardiotoxicity in cancer patients.

AUTHOR CONTRIBUTIONS

Qingling Zhang: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing—original draft (equal); writing—review and editing (equal). **Yunpeng Zhang**: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing—review and editing (equal). **Bingxin Xie**: Formal analysis (supporting); investigation (supporting); methodology (supporting); validation (supporting). **Daiqi Liu**: Formal analysis (supporting); investigation (supporting); methodology (supporting); validation (supporting). **Yueying Wang**: Formal analysis (supporting); methodology (supporting); validation (supporting). **Zandong Zhou**: Formal analysis (supporting); methodology (supporting); validation (supporting). **Yue Zhang**: Methodology (supporting); validation (supporting). **Emma King**: Writing—review and editing (supporting). **Gary Tse**: Writing—review and editing (supporting). **Tong Liu**: Conceptualization (lead); funding acquisition (lead); project administration (lead); supervision (lead).

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None.

CONFLICT OF INTEREST STATEMENT

Professor Tong Liu is a member of the *Cancer Innovation* Editorial Board. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The protocol for this study and the use of animals were approved by the Experimental Animal Administration Committee of Tianjin Medical University (TMUaMEC 2019004). Experiments were carried out following the guidelines of the National Institutes of Health of the United States and in line with the 3R principles.

INFORMED CONSENT

Authors declare informed consent was not needed for this study.

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