

LncRNA SUMO1P3 aggravates doxorubicin-induced cardiomyocyte apoptosis by targeting miR-93-5p / Bin1

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Research article

Keywords: SUMO1P3, miR-93-5p, Bin1, DOX, cardiomyocytes

Posted Date: March 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-167434/v2>

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Abstract

Background: Non-coding RNA plays a critical role in myocardial apoptosis induced by doxorubicin (DOX). However, the specific function of Long noncoding RNA (lncRNA) small ubiquitin-like modifier 1 pseudogene 3 (SUMO1P3) is unclear. The purpose of this study was to determine the role of lncRNA SUMO1P3 in myocardial apoptosis induced by DOX.

Methods: QRT-PCR were used to detect the expression levels of SUMO1P3 and miR-93-5p in DOX-treated primary cardiomyocytes and rat models. QRT-PCR and Western blot were used to detect the expression levels of Bin1 in DOX-treated primary cardiomyocytes and rat models. The relationship between SUMO1P3, miR-93-5p and Bin1 was analyzed using bioinformatics analysis and Luciferase reporter assay. The effects of DOX on the viability and apoptosis of cardiomyocytes were evaluated by flow cytometry and CCK-8. The effects of SUMO1P3 on cardiomyocyte apoptosis were analyzed by TUNEL staining and echocardiography.

Results: In DOX-treated primary cardiomyocytes and rat models, the expression levels of SUMO1P3 and Bin1 were significantly increased, while the expression levels of miR-93-5p were significantly reduced. MiR-93-5p was a direct target gene of SUMO1P3, and Bin1 was a direct target gene of miR-93-5p. In addition, miR-93-5p reversed the protective effect of SUMO1P3 knockout on cardiomyocytes by inhibiting the expression of Bin1.

Conclusions: SUMO1P3 inhibited DOX-induced cardiomyocyte apoptosis through miR-93-5p/Bin1 axis.

Introduction

Doxorubicin (DOX) is a clinically effective and widely used antitumor drug [1, 2]. However, its clinical application is limited by its cardiotoxicity [3]. The cardiotoxicity of DOX can be divided into acute toxicity and chronic toxicity according to the course of the disease [4]. Acute toxicity is often manifested as acute multiple organ failure induced by a small dose of DOX. However, the chronic toxicity of DOX is more common in the clinic [5]. Because of the strong affinity between DOX and cardiomyocytes, DOX is easy to accumulate in the heart and produce cardiotoxicity. Long term use of drugs causes damage to the function of myocardial cells, leads to irreversible myocardial damage and dysfunction, eventually induces dose-dependent and time-dependent congestive heart failure [6]. The end-stage heart failure for cancer patients seriously affects the survival rate [7]. The mechanism has always been a research hotspot, but the exact mechanism needs to be further explored. At present, the main view is that the toxicity of DOX is mainly reflected in the induction of oxidative stress and lipid peroxidation in cardiomyocytes, which affect the synthesis of nucleic acid and protein [8]. Among them, cardiomyocyte apoptosis may be a critical role of DOX-induced cardiotoxicity [9]. DOX can lead to cardiac dysfunction, and eventually develop into severe heart failure or even death. Free radical injury, mitochondrial injury, abnormal energy metabolism, calcium overload and apoptosis are all involved in DOX induced myocardial injury. At present, it is considered that the cardiotoxicity of DOX may be the result of many factors, which are

related to each other and promote the development of cardiotoxicity [10]. Therefore, it is urgent to develop new prevention strategies for DOX-induced cardiotoxicity.

Long noncoding RNAs (lncRNAs) are involved in cardiac development and closely related to heart disease [11, 12]. LncRNAs are RNAs that are longer than 200 bp and do not have protein encoding functions [13]. Given the critical roles of LncRNAs in many important regulatory processes, such as genomic imprinting, transcriptional activation, transcriptional interference, chromatin modification, this suggests a tremendous influence of LncRNAs in cardiovascular diseases [14]. Recent studies of lncRNAs in the cardiac system have mainly focused on its role in heart injury and remodeling, leukocyte and inflammation [15]. For example, it is found that lncRNA GASL1 is downregulated in chronic heart failure (CHF). The overexpression of lncRNA GASL1 may improve CHF by inhibiting the inactivation of TGF- β 1 and inhibiting cardiomyocyte apoptosis [16]. In particular, lncRNA SUMO1P3 is a recently discovered new lncRNA and has been indicated as an oncogenic lncRNA in bladder cancer and breast cancer [17, 18]. However, there haven't been any studies on the biological function of SUMO1P3 on DOX induced cardiac cells.

Accumulating evidence demonstrates that lncRNAs serve as competing endogenous RNAs (ceRNAs) through competitive binding to miRNAs [19]. miRNA is widely involved in cell differentiation, disease, repair and apoptosis. A variety of miRNAs can be expressed in mammalian cardiac tissue, which play a critical role in the progress of cardiac diseases, including cardiac hypertrophy, arrhythmia, myocardial infarction, myocardial fibrosis, myocardial cell apoptosis, heart failure and myocardial apoptosis [20-22]. In particular, recent studies have found that miR-93-5p plays an important role in the pathological processes of various cancer via regulating the expression of related genes [23]. For example, miR-93-5p inhibits the cellular migration of breast cancer cells [24] and promotes gastric cancer metastasis [25]. Previous study has shown that miR-93 inhibits the oxygen-glucose deprivation/reoxygenation-induced the cardiomyocyte apoptosis [26]. However, the role of miR-93-5p in DOX-induced cardiomyocyte apoptosis and its target are not clear. Based on the prediction results of Starbase v2.0, one of the putative targets of miR-93-5p is Bin1. Bin1 is a N-terminal binding protein of c-myc protein, which is the only ligand protein with anti-cancer function [27]. It has been shown that Bin1 expression is low or absent in breast cancer, liver cancer, bladder cancer and colon cancer [28, 29].

Based on the aforementioned literature, the interactions between lncRNAs, miRNA and Bin1 are critical regulators in cell cycle progression and relevant diseases. Thus, we hypothesize that lncRNA SUMO1P3 has protective effect on DOX-induced myocardial injury through the miR-93-5p/Bin1 axis. This provides a theoretical basis for expanding the biological role of SUMO1P3 and clinical protection of DOX-induced myocardial injury.

Materials And Methods

Primary Cardiomyocyte Cultures

Postnatal Wistar rats younger than 12 h were chosen and disinfected using 75% ethanol. The hearts were separated and soaked in 1% PBS to remove the blood. Then, the ventricles were selected and cut into 1 mm³ blocks. The tissue blocks were digested with 0.25% trypsin-EDTA (Gibco, USA) at 37 °C for 8 min, and the upper layer suspension was discarded. Then, the remaining tissues were continued to be digested with 0.25% trypsin-EDTA at 37 °C for 8 min, and the upper layer suspension was collected. Then, the action of trypsin was terminated by the addition of the same amount of culture medium (10% fetal bovine serum, 1% L-glutamine, 1% penicillin-streptomycin solution and 88% DMEM). The remaining tissues were continued to be digested 3-5 times using the aforementioned steps until all the tissues were digested. The collected suspension was centrifuged at 1,000 rpm for 10 min. The cells were plated at a cell density of 5 × 10⁵ cells/mL on the 0.1 mg/mL poly-D-lysine-coated 15-mm confocal dishes. Afterwards, the medium was changed every two days. The experimental results confirmed that the isolated cells expressed cardiac- α -actin, and successfully established the model of cultured cardiomyocytes *in vitro*.

Cell culture

HEK293T cells for the luciferase reporter assay were purchased from the Institute of Biochemistry and Cell Biology (Shanghai). All cells were cultured in DMEM Medium (Gibco, USA) supplementing with 10% fetal bovine serum (FBS) (Invitrogen), and 1% penicillin/streptomycin at 37°C with 5% CO₂.

Cell transfection

PcDNA3.1-SUMO1P3 (SUMO1P3) (5'-CAAUCAACUCUGAGAUCATT-3'), SUMO1P3 (si-SUMO1P3) siRNA, miR-93-5p mimic (Hsa-miR-93-5p:5'-UAGCAG-CACGUAAAUAUUGGCG-3'), miR-93-5p agomir, si-Bin1 (5'-UAGCAGCACAUAAUGGUUUGUG-3') and their corresponding controls (GenePharma, Shanghai, China) were synthesized by GenePharma (Shanghai China). Oligonucleotides were transfected into the cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

***In vivo* studies**

All animal studies were approved by First Affiliated Hospital of Xi'an Jiaotong University Animal Protection and Committee. DOX at a dose of 8 mg/kg was injected intraperitoneally into the rats at 3 week intervals (cumulative dose of 24 mg/kg). Rats in the control group (sham operation group, n = 10) were injected intraperitoneally with saline. The parameters of echocardiography were analyzed by VEVO 770 (VisualSonics Inc, Toronto, Canada). LVFS (left ventricular fractional shortening) and LVEF (Left ventricular ejection fraction) were analyzed by linear sensor (Minuo, Shenzhen, China).

Adenovirus gene delivery

Recombinant adenovirus containing mouse SUMO1P3 shRNA (Ad- SUMO1P3-shRNA), 7 days before DOX induction, adenovirus was intratracheally dripped into rats. Control adenovirus (Ad-GFP) was injected into the control group. MiR-93-5p mimic and its NC were purchased from GenePharma (Shanghai, China). 50

µg miR-93-5p mimic or its negative control was dissolved in 50 µl of sterile double distilled water, and 50 µl of glucose solution was added. Then, the tail vein of rats was injected with 200 µl working solution.

Dual luciferase reporter gene assay

The wild-type (WT) 3'-UTR of SUMO1P3 cDNA was synthesized by PCR and cloned into pMIR-REPORT luciferase to generate WT- SUMO1P3 3'-UTR. Based on the mutants of SUMO1P3 3'- UTR, the resulting vectors were named as MUT-SUMO1P3 3'-UTR. These vectors (pMIR-REPORT plasmid, WT-SUMO1P3 3'-UTR or MUT-SUMO1P3 3'-UTR) and miR-93-5p mimic or NC was transiently transfected into HEK293 cells by Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). After 48 h, luciferase activity was analyzed by a dual luciferase reporter gene assay system (Promega, Madison, WI, USA).

RNA extraction and quantitative real-time PCR

Total RNA in tissues and cells was extracted using TRIzol reagent (Biosntech, Beijing, China). The quality of RNA was analyzed using NanoDrop 1000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). SYBR-Green (Takara Biotechnology, Co., Lt. (Dalian, China) was used in by qRT-PCR. Amplification was performed by ABI 7,500 real-time PCR system. A qScript microRNA cDNA synthesis kit (Quantabio, Beverly, CA, USA) was used for cDNA synthesis. The expression levels of SUMO1P3 and miR-93-5p were calculated using the $2^{-\Delta\Delta CT}$ method. The expression levels of miRNA were standardized by U6. The expression levels of lncRNA were standardized by GAPDH. The primers used for qRT-PCR analysis were listed in Table 1 (n=3).

Table 1. Primers used in this study.

Name	Sequence (5'-3')
SUMO1P3	forward: GTAGCCTTCTGAAACGGCAATTG
	reverse: AGTGCAAGTGTGGGAGATTCATC
Bin1	forward: ACCCATCGAGCACCTAGAGA
	reverse: CTGAGGATTGGCTATCGTGCC
miR-93-5p	forward: GCAGTGGCCTTAGCGGACAC
	reverse: CAGATTCTTAGAACGAGGAC
U6	forward: AGAGAAGGTTAGCATGGCCCCTG
	reverse: AGTGCAGGGTCCGAGGTATT
GAPDH	forward: CCAAGGTCATCCATGACAAC
	reverse: GCTTCACCACCTTCTTGATG

Western blot

The cells were harvested and washed with 1 × PBS, and then we used 2 × SDS loading buffer to lyse cells. The lysates were boiled at 95°C for 10 min. The solution was subject to centrifuge at 12,000 rpm for 1 min. About 60 ug of total protein (10 -15 ul) was loaded onto SDS-PAGE gel and resolved at 120 V for 0.5–1 h. After that, the proteins were transferred to PVDF membrane at 300 mA for 2–3 h. The membrane was blocked with 5% non-fat milk in 1 × TBST for 1 h at room temperature, and then the membrane was incubated with proper primary antibodies at 4°C, overnight. The following day, the membrane was washed with 1 × TBST for 3 times, 10 min each time. The membrane was incubated with secondary antibody at room temperature for 1 h. Finally, the membrane was incubated with ECL and then exposed using Bio-Rad ChemiDoc Touch Imaging System. The following antibodies were used in this study: anti-Bin1 (1: 1000, Youliante, Shanghai, China) and anti-GAPDH antibodies (1: 1000, Youliante, Shanghai, China) overnight. An anti-rabbit secondary antibody (1: 1000, Youliante, Shanghai, China). Results were visualized with using the Supersignal West Dura Substrate (Pierce). After that, the relative protein expression was expressed as the ratio of the gray value of the target band / GAPDH band.

Cell Counting Kit-8 Analysis

The Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Japan) was used to assess the rate of cell proliferation. In brief, primary cardiomyocytes were plated in 96-well plates at approximately 2000 cells per well with 200 µL of culture medium and were treated with DOX at different concentrations when needed. After 24 hours, 10 µl of CCK8 solution was applied to each well, and the plates were incubated for 1 h at 37 °C. Finally, the absorbance values at 450 nm were determined using a microplate reader (Multiskan, Thermo, USA) with a reference wavelength of 650 nm. All of the experiments were conducted at least in triplicate.

Annexin V-FITC/PI double-labeled flow cytometry

Cell apoptosis was analyzed by flow cytometry using Annexin V-FITC apoptosis detection kit (BD Biosciences; San Jose, CA, USA) according to the manufacturer's protocols. Primary cardiomyocytes following transfection were collected, washed with cold PBS and stained with binding buffer containing Annexin V-FITC and propidine iodide (PI) at 4°C under darkness for 15 min. Finally, cells were recorded using flow cytometry (Beckman Coulter, Fullerton, CA, USA).

TUNEL analysis

The TUNEL method was used to analyze the apoptotic index of myocardial cells. The nuclei of positive apoptotic cells were brown-yellow granules. Five 400 high-power fields were randomly observed. The apoptotic index (AI), $AI (\%) = (\text{number of apoptotic positive nuclei} / \text{total counted nuclei}) \times 100\%$, was calculated.

Statistical methods

Data are expressed as mean ± SEM. Statistical analysis of the results was performed with GraphPad Prism version 5.0 (GraphPadSoftware Inc., San Diego, CA, USA). Student's t-test was used for two group

comparison. One-way ANOVA followed by Dunnett t-test was used for multiple group comparison. $P < 0.05$ was considered statistically significant.

Result

SUMO1P3 was highly expressed in doxorubicin stimulated primary cardiomyocyte

As shown in Fig. 1A and 1B, DOX gradually reduced the cell viability of primary cardiomyocyte cells with the increase in the dose and duration ($P < 0.01$). Then, the expression level of SUMO1P3 was analyzed in the cells after DOX treatment. The expression level of SUMO1P3 was increased significantly with the increase of the dose and duration of DOX action ($P < 0.01$, Fig.1C and 1D). These results suggest that SUMO1P3 plays a key role in myocardial cell apoptosis induced by DOX.

SUMO1P3 knockdown inhibited myocardial apoptosis

To further analyze whether SUMO1P3 was involved in adriamycin-induced apoptosis of myocardial cells. DOX significantly inhibited the activation of primary cardiomyocytes and induced the apoptosis of primary cardiomyocytes contrasted with the Control group ($P < 0.01$). The $2^{-\Delta\Delta Ct}$ values of si- NC and si-SUMO1P3 was (18.19 ± 7.94) and (33.42 ± 2.64) respectively. Contrasted with the DOX-treated group, the viability of primary cardiomyocytes was significantly raised, and the apoptosis of primary cardiomyocytes was inhibited after DOX treatment in the si-SUMO1P3 group ($P < 0.01$, Fig. 2A and 2B). As shown in Fig. 2C, compared to the control group, DOX treatment significantly increased the expression levels of Bax and C-Caspase-3 protein ($P < 0.01$) and inhibited the expression level of Bcl-2 ($P < 0.01$) in the si-NC group. Contrasted with the DOX-treated group, transfection with si-SUMO1P3 significantly inhibited the Dox-induced Bax and C-Caspase-3 protein expression ($P < 0.01$), and restored the Bcl-2 protein expression level in primary cardiomyocytes ($P < 0.01$, Fig. 2C). These results indicate that si-SUMO1P3 inhibits the apoptosis of primary cardiomyocytes.

SUMO1P3 functions as a ceRNA of miR-93-5p in primary cardiomyocytes

Next, the potential mechanism of SUMO1P3 regulating DOX-induced cytotoxicity was explored. Starbase v2.0 online prediction tool was used and predicted that MiR-93-5p was a potential target of SUMO1P3 (Fig.3A). To confirm whether miR-93-5p directly binds to SUMO1P3, we performed a dual-luciferase reporter assay. SUMO1P3 containing the wild type or mutant type putative miR-93-5p binding site was inserted into a reporter vector that was cotransfected with miR-93-5p mimics into cells. The results demonstrated that miR-93-5p overexpression significantly reduced the luciferase activity of a reporter vector containing the wild type SUMO1P3 ($P < 0.01$) (Fig. 3B). However, miR-93-5p overexpression showed no obvious effect on the luciferase activity of the reporter vector containing the mutant SUMO1P3 (Fig. 3B). As shown in Fig. 3C, the expression level of SUMO1P3 was reduced in the si-SUMO1P3 group while increased in the SUMO1P3 mimic group, indicating high efficiency of transfection. Compared to the si-NC group, the expression level of miR-93-5p was significantly increased in the si-SUMO1P3 group ($P < 0.05$), while significantly reduced after the addition of SUMO1P3 ($P < 0.05$) (Fig.

3C). In addition, the expression level of miR-93-5p in the DOX group was significantly reduced contrasted with the Control group ($P < 0.05$, Fig. 3D). In summary, these results demonstrate that SUMO1P3 targets and negatively regulates the expression of miR-93-5p.

SUMO1P3 sponged and sequestered miR-93-5p to upregulate Bin expression

We predicted that Bin1 might be a potential target for miR-93-5p through online prediction tool Starbase v2.0 (Fig. 4A). To confirm whether miR-93-5p directly binds to Bin1 3'-UTR, dual-luciferase reporter assay was performed. Bin1 3'-UTR containing the wild type or mutant type putative miR-93-5p binding site was inserted into a reporter vector that was cotransfected with miR-93-5p mimics into cells. The results demonstrated that miR-93-5p overexpression significantly reduced the luciferase activity of a reporter vector containing the wild type Bin1 3'-UTR ($P < 0.01$) (Fig. 4B). However, miR-93-5p overexpression showed no obvious effect on the luciferase activity of the reporter vector containing the mutant Bin1 3'-UTR (Fig. 4B). Transfection of miR-93-5p mimic significantly increased the expression level of miR-93-5p, which was in turn reduced by co-transfection of SUMO1P3 (Fig. 4C). This result was consistent with previous data in Fig. 3C. Compared with the NC group, the expression level of Bin1 was significantly decreased in the miR-93-5p mimic group ($P < 0.01$), while it was significantly increased in the SUMO1P3 overexpression group ($P < 0.01$). Co-transfection of SUMO1P3 with miR-93-5p reversed SUMO1P3-induced expression of Bin1 ($P < 0.01$, Fig. 4C). The $2^{-\Delta\Delta Ct}$ values of miR-NC and miR-93-5p mimic was (15.04 ± 4.58) and (27.32 ± 5.71) , respectively. As shown in Fig. 4C and 4D, contrasted with the miR-NC group, the expression level of Bin1 mRNA and protein was significantly reduced in the miR-93-5p mimic group ($P < 0.01$), and the expression levels of Bin1 gene and protein were significantly increased in the SUMO1P3 overexpression group ($P < 0.01$) (Fig. 4C-4D). Co-transfection of SUMO1P3 with miR-93-5p reversed the SUMO1P3-induced expression of Bin1 ($P < 0.01$, Fig. 4C) ($P < 0.01$) (Fig. 4C-4D). In addition, the expression level of Bin1 in the DOX treatment group was significantly raised compared with the Control group ($P < 0.01$, Fig. 4E). In summary, these results demonstrate that SUMO1P3 increases the expression of Bin1 by acting as a sponge for miR-93-5p.

SUMO1P3 knockdown inhibited myocardial apoptosis via miR-93-5p/Bin1 axis *in vitro*

Next, the mechanism of action of SUMO1P3 in adriamycin-induced cardiomyocyte apoptosis was further analyzed. DOX significantly inhibited the proliferation of primary cardiomyocytes contrasted with the control group ($P < 0.01$), and cell viability of primary cardiomyocytes was significantly raised in the si-SUMO1P3 group, miR-93-5p mimic group, and si-Bin1 group ($P < 0.01$), while co-transfection of si-SUMO1P3 with miR-93-5p or si-SUMO1P3 and si-Bin1 reversed the effect of si-SUMO1P3 on the viability of primary cardiomyocytes ($P < 0.01$, Fig. 5A and 5B). As shown in Fig. 5C, DOX significantly promoted the apoptosis of primary cardiomyocytes ($P < 0.01$). Apoptosis of primary myocardial cells was significantly inhibited in the si-SUMO1P3 group, miR-93-5p overexpression group and si-Bin1 group ($P < 0.01$), while co-transfection of si-SUMO1P3 with miR-93-5p or si-SUMO1P3 and si-Bin1 reversed the effect of si-SUMO1P3 on primary cardiomyocyte apoptosis ($P < 0.01$). These results indicate that SUMO1P3 inhibits primary cardiomyocyte apoptosis through miR-93-5p / Bin1.

SUMO1P3 knockdown improved myocardial function through regulating miR-93-5p *in vivo*

Four weeks after the first injection of DOX in rats, organ samples were collected and cardiac function was measured by echocardiography. As shown in Fig.6A and 6B, si-SUMO1P3 significantly inhibited the expression levels of SUMO1P3 and Bin1 ($P < 0.01$), while si-SUMO1P3 significantly increased the expression level of miR-93-5p in mouse heart tissues ($P < 0.01$), co-transfection of si-SUMO1P3 with miR-93-5p reversed the effects of si-SUMO1P3 on SUMO1P3, Bin1 and miR-93-5p expression levels ($P < 0.01$). Contrasted with the Control group, LVEF and LVFS scores were significantly reduced after DOX treatment ($P < 0.01$), while LVEF and LVFS scores were significantly raised in the si-SUMO1P3 group and miR-93-5p overexpression group ($P < 0.01$). Co-transfection of si-SUMO1P3 with miR-93-5p was able to reverse the effect of si-SUMO1P3 on LVEF and LVFS scores ($P < 0.01$, Fig. 6C and 6D). As The TUNEL staining results showed that the apoptosis rate in the DOX group was significantly increased ($P < 0.01$), the apoptosis rate was significantly reduced in the si-SUMO1P3 group and the miR-93-5p overexpression group ($P < 0.01$), while co-transfection of si-SUMO1P3 and miR-93-5p reversed the effect of si-SUMO1P3 on the apoptosis rate ($P < 0.01$, Fig. 6E). These results indicate that si-SUMO1P3 relieves myocardial apoptosis via miR-93-5p/Bin1 axis.

Discussion

Doxorubicin (DOX) is often used to treat hematological malignancies and solid tumors[30]. However, it has been found in clinical studies that doxorubicin has a strong affinity with cardiomyocytes[31, 32]. Although cardiomyocytes cultured *in vitro* lack the persuasiveness of animal experiments in the observation of end events, they reduce bias factors and are more conducive to the study of cell mechanism. These experiments explored the potential mechanisms of DOX cardiotoxicity by establishing cardiomyocyte apoptosis model *in vitro*.

The role of long-chain non-coding RNA (lncRNA) has attracted widespread attention [33]. lncRNAs have been reported in nervous system disorders, metabolic diseases, reproductive development and cardiovascular diseases [34]. Recent study has confirmed that lncRNA may play an important role in cardiac regeneration and repair as a potential target of treatment [35]. For example, studies have found that lncRNA Carl inhibits mitochondrial fission and cardiomyocyte apoptosis and reduces ischemia-reperfusion injury[36]. So far, the mechanism of lncRNA on DOX-induced cardiotoxicity is still largely unknown. lncRNA SUMO1P3 is a recently discovered lncRNA, and studies has found that SUMO1P3 is oncogenic. Zhang et al. found that SUMO1P3 was significantly up-regulated in bladder cancer tissues, and knockdown of SUMO1P3 inhibited bladder cancer cell proliferation/migration inhibition and induced bladder cancer cell apoptosis [37]. Similarly, Liu et al. found that SUMO1P3 expression was higher in breast cancer tissues and the high levels of SUMO1P3 expression associated significantly with tumor progression and poor survival of breast cancer patients. Moreover, knockdown of SUMO1P3 suppressed proliferation, migration and invasion of breast cancer cells [18]. Altogether, these studies suggest that SUMO1P3 functions as an oncogenic lncRNA in some cancer cells. In our present study, we found that

with the increase of the dose and duration of DOX, the expression level of SUMO1P3 was raised. Moreover, si-SUMO1P3 inhibited the apoptosis of primary cardiomyocytes.

A subset of miRNAs has been shown to be highly and specifically expressed in the heart muscle, including miRNA-378, miRNA-208, miRNA-499 and miRNA-22 [38, 39]. For example, it has been found that overexpression of miRNA-133a in cardiomyocytes significantly reduces H₂O₂ induced cardiomyocyte apoptosis, indicating that miRNA plays a critical role in the mechanism of anti-cardiomyocyte apoptosis [40]. miR-93-5p has different roles in different diseases [41]. For example, miR-93-5p enhances the growth of HUVECs [42]. Our present study finds that miR-93-5p was a potential target of SUMO1P3. The expression level of miR-93-5p in the DOX group was significantly reduced. The expression level of miR-93-5p in the si-SUMO1P3 group was reduced, while the expression levels of LVEF and LVFS were raised, and the apoptosis rate was reduced in the si-SUMO1P3 group and miR-93-5p group. Co-transfection of si-SUMO1P3 with miR-93-5p reversed the effect of si-SUMO1P3 on LVEF, LVFS score and apoptosis. These results demonstrated SUMO1P3 exerted their biological functions by miR-93-5p.

Bin1 is reported to bind to the Myc binding domain (MBD) site at the N-terminal of c-MYC [43], which is a ligand protein with anti-cancer characteristics and has the characteristics of tumor suppressor gene, and is likely to participate in the downregulation of cell growth [44]. Bin1 is widely expressed in the normal body cells [45]. The proliferation, metastasis and invasion of malignant cells to surrounding tissues may be caused by the absence of Bin1. The overexpression of Bin1 inhibits the growth or infiltration of tumor cells, apoptosis and malignant transformation [46]. Bin1 plays a part in the occurrence of many diseases, including cardiovascular and cerebrovascular diseases, etc. [47]. This study found that Bin1 is a potential target of miR-93-5p. Viability of primary cardiomyocytes in the si-SUMO1P3 group, miR-93-5p mimic group, and si-Bin1 group was raised, and apoptosis was reduced. Co-transfection of si-SUMO1P3 with miR-93-5p or si-SUMO1P3 and si-Bin1 reversed the effects of si-SUMO1P3 on the viability and apoptosis of primary cardiomyocytes. These results indicate that SUMO1P3 inhibits primary cardiomyocyte apoptosis through miR-93-5p/Bin1.

Conclusion

Si-SUMO1P3 protected myocardial cells from DOX-induced damage through miR-93-5p/Bin1, which is of great significance for the treatment of DOX-induced heart damage.

Declarations

Acknowledgements

Not applicable.

Author Contributions

Aiqun Ma designed the study. Xuefeng Lin carried out experiments and wrote the manuscript, Aiqun Ma

revised the paper, Xuefeng Lin collected patient specimens and related information and contributed to analysing the data. All authors reviewed the results and approved the final version of the manuscript.

Funding

This work was supported by Inner Mongolia Natural Science Foundation (No. 2016MS08101).

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author, Aiqun Ma. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

No applicable.

Competing interests

The authors report no conflicts of interest for this work and have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Abbreviations

DOX: doxorubicin ;

lncRNA: Long noncoding RNA;

SUMO1P3: small ubiquitin-like modifier 1 pseudogene 3 ;

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Figures

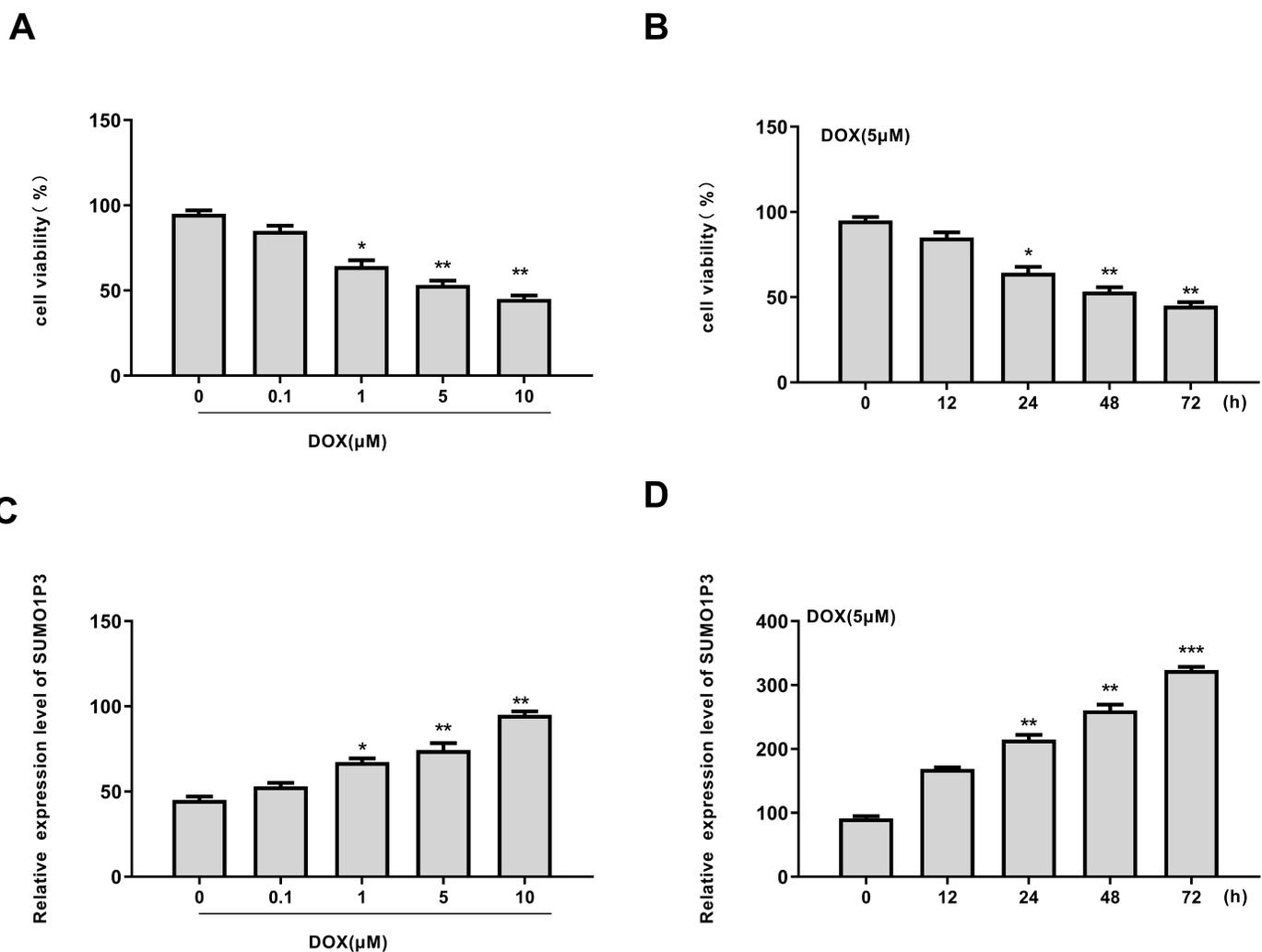


Figure 1

SUMO1P3 expression level in primary cardiomyocytes. (A) Cell viability was determined after stimulating cells with DOX (0.1 μM -10 μM) for 24 hours. (B) Cell viability was determined after stimulating cells with

DOX (5 μ m) from 12 to 72 hours. (C) SUMO1P3 expression was measured after stimulating cells with different concentrations of DOX (0.1 - 10 μ M) for 24 hours. (D) The expression level of SUMO1P3 was measured after stimulating cells with DOX (5 μ m) from 12 to 72 hours. N = 3. ** p < 0.01.

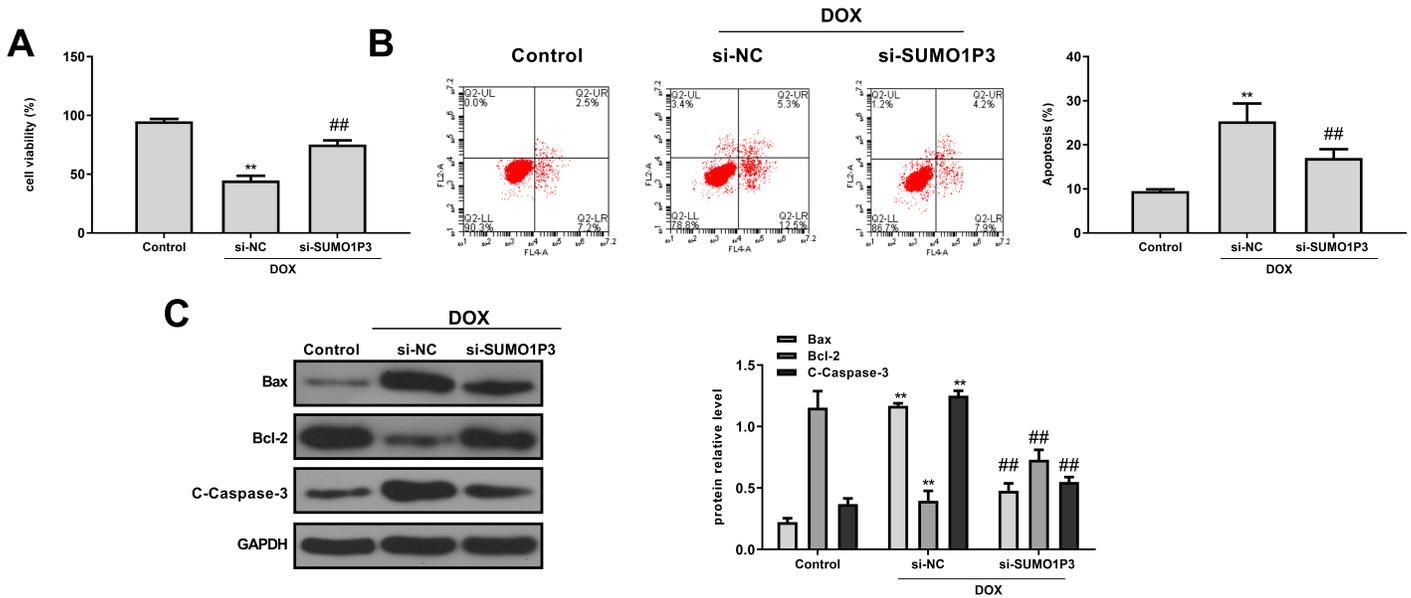


Figure 2

The role of SUMO1P3 in DOX-induced cardiomyocyte apoptosis. (A) Viability of primary cardiomyocytes. (B) Apoptosis rate of primary cardiomyocytes. (C) Protein expression levels of caspase-3, Bcl-2 and Bax in primary cardiomyocytes. N = 3. ** P < 0.01 vs Control group; ## P < 0.01 vs si-NC group.

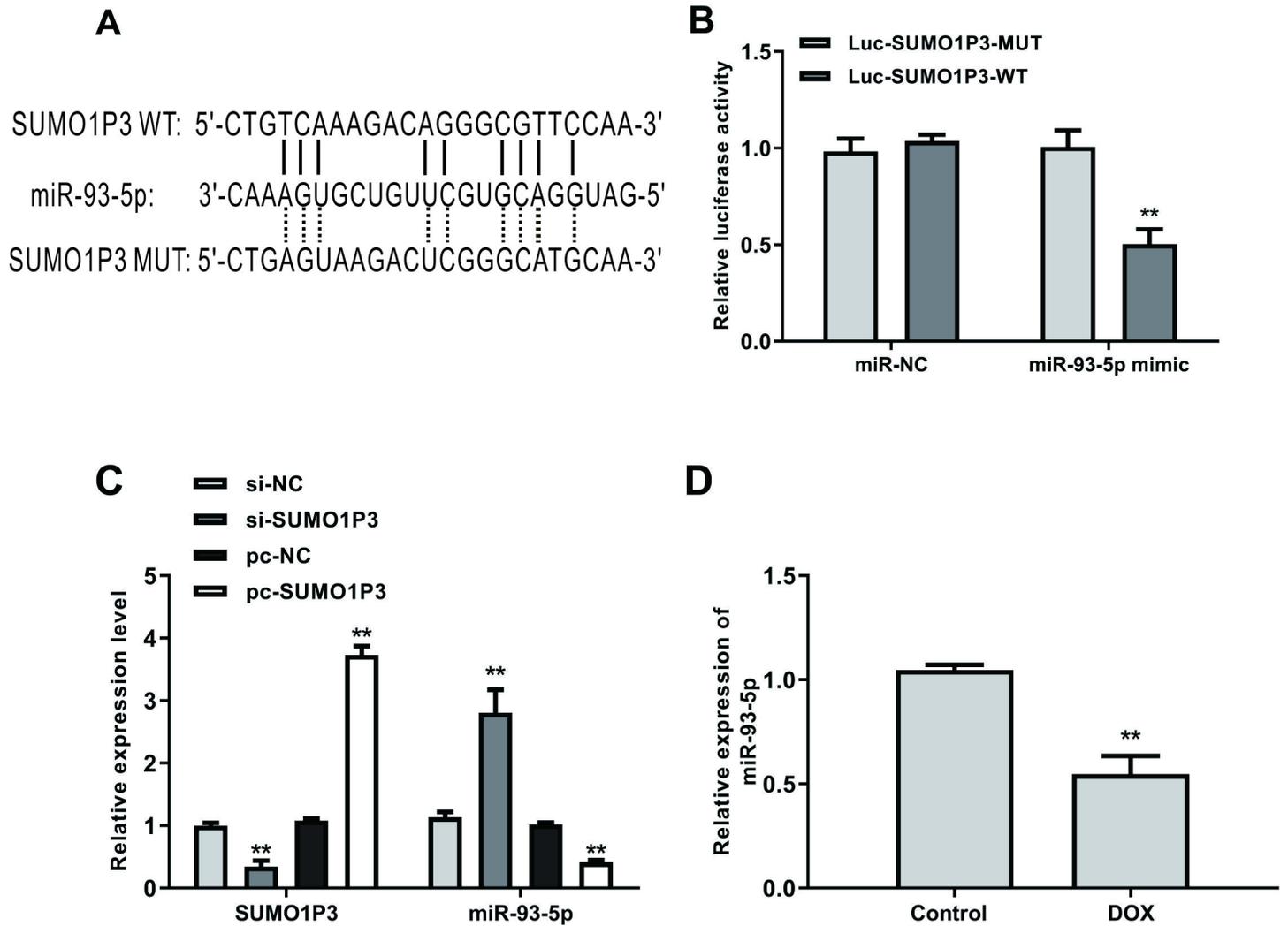
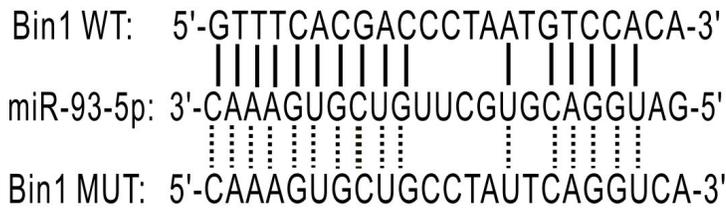
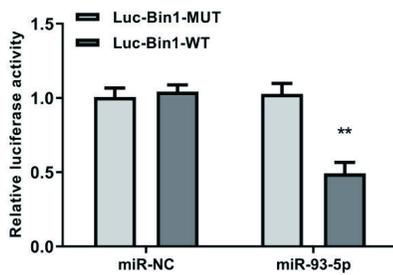
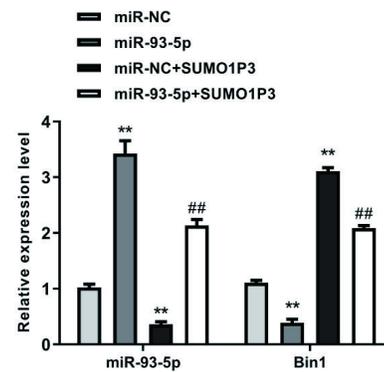
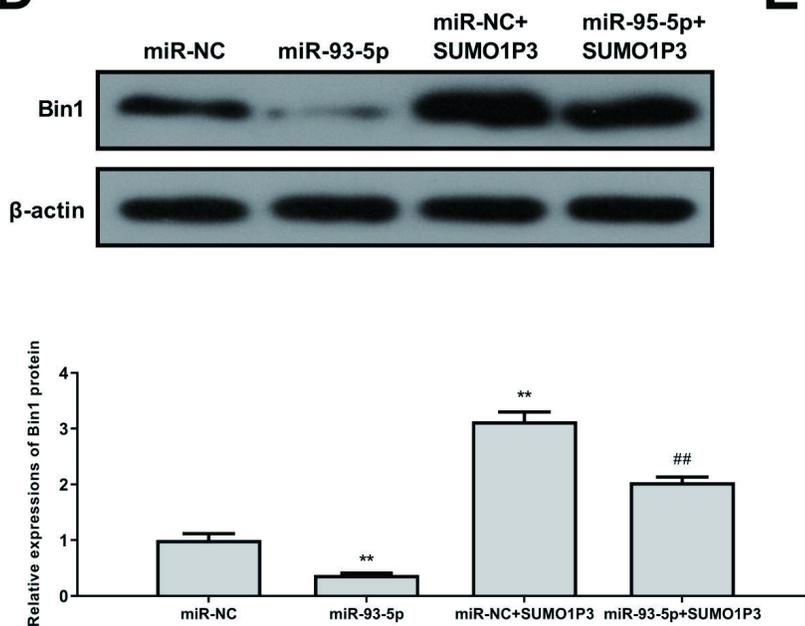
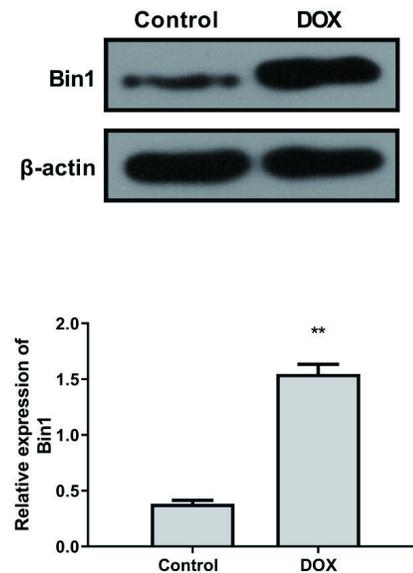


Figure 3

SUMO1P3 negatively regulates the expression of miR-93-5p. (A) Putative binding sites for SUMO1P3 and miR-93-5p. (B) Luciferase activity in HEK293 cells co-transfected with miR-93-5p mimic and SUMO1P3-WT or SUMO1P3-Mut vector. (C) The expression levels of SUMO1P3 (left) and MiR-93-5p (right) in primary cardiomyocytes transfected with four different vectors: si-NC, si-SUMO1P3, NC and SUMO1P3. (D) miR-93-5p gene expression level in primary cardiomyocytes with DOX treatment. N = 3. ** P < 0.01.

A**B****C****D****E****Figure 4**

SUMO1P3 up-regulated Bin1 expression through miR-93-5p. (A) Putative binding sites for miR-93-5p and Bin 1 3'-UTR. (B) Luciferase activity in HEK293 cells co-transfected with miR-93-5p mimic and Bin 1 3'-UTR-WT or Bin 1 3'-UTR-Mut vector. (C) Relative expression level of Bin1 mRNA in primary cardiomyocytes that were transfected with four different vectors (from left to right): miR-93-5p-NC, miR-93-5p mimic, miR-93-5p-NC + SUMO1P3, and miR-93-5p mimic + SUMO1P3. (D) Relative expression level of Bin1 protein in

primary cardiomyocytes transfected with four different vectors (from left to right): miR-93-5p-NC, miR-93-5p mimic, miR-93-5p-NC + SUMO1P3, and miR-93-5p mimic + SUMO1P3. (E) Bin 1 protein expression level in DOX. N = 3. ** P < 0.01 vs Control group; ## P < 0.01 vs miR-93-5p group.

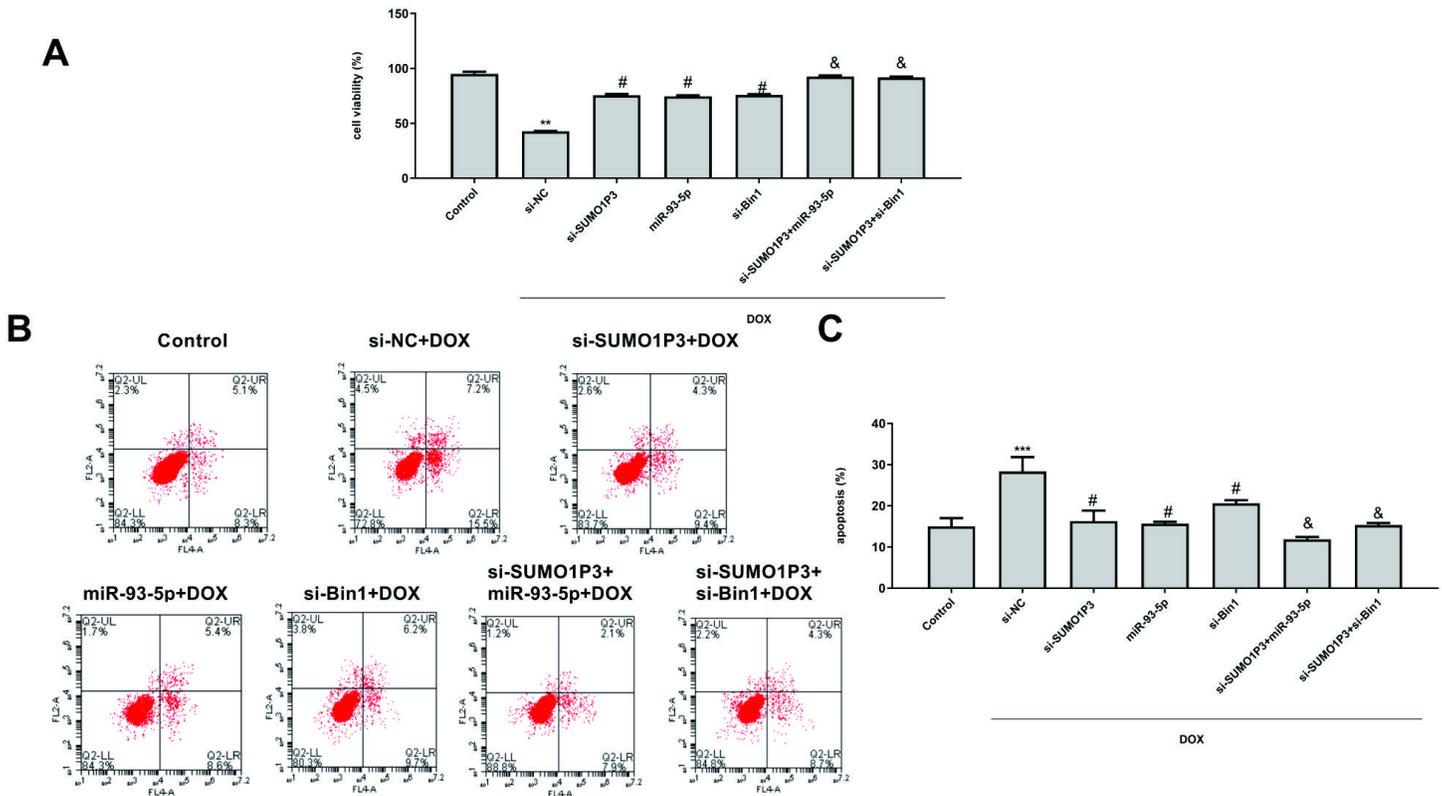


Figure 5

The biological role of SUMO1P3 in DOX-induced myocardial apoptosis. (A, B) Cell viability of primary myocardium in si-NC, si-SUMO1P3 and miR-NC, miR-93-5p mimics or si-Bin1. (C) Apoptosis rate of primary cardiomyocytes transfected with si-NC, si-SUMO1P3 and miR-NC, miR-93-5p mimics or si-Bin1. N = 3. ** P < 0.01 vs Control group; ## P < 0.01 vs si-NC group, & P < 0.05 vs si-SUMO1P3 group.

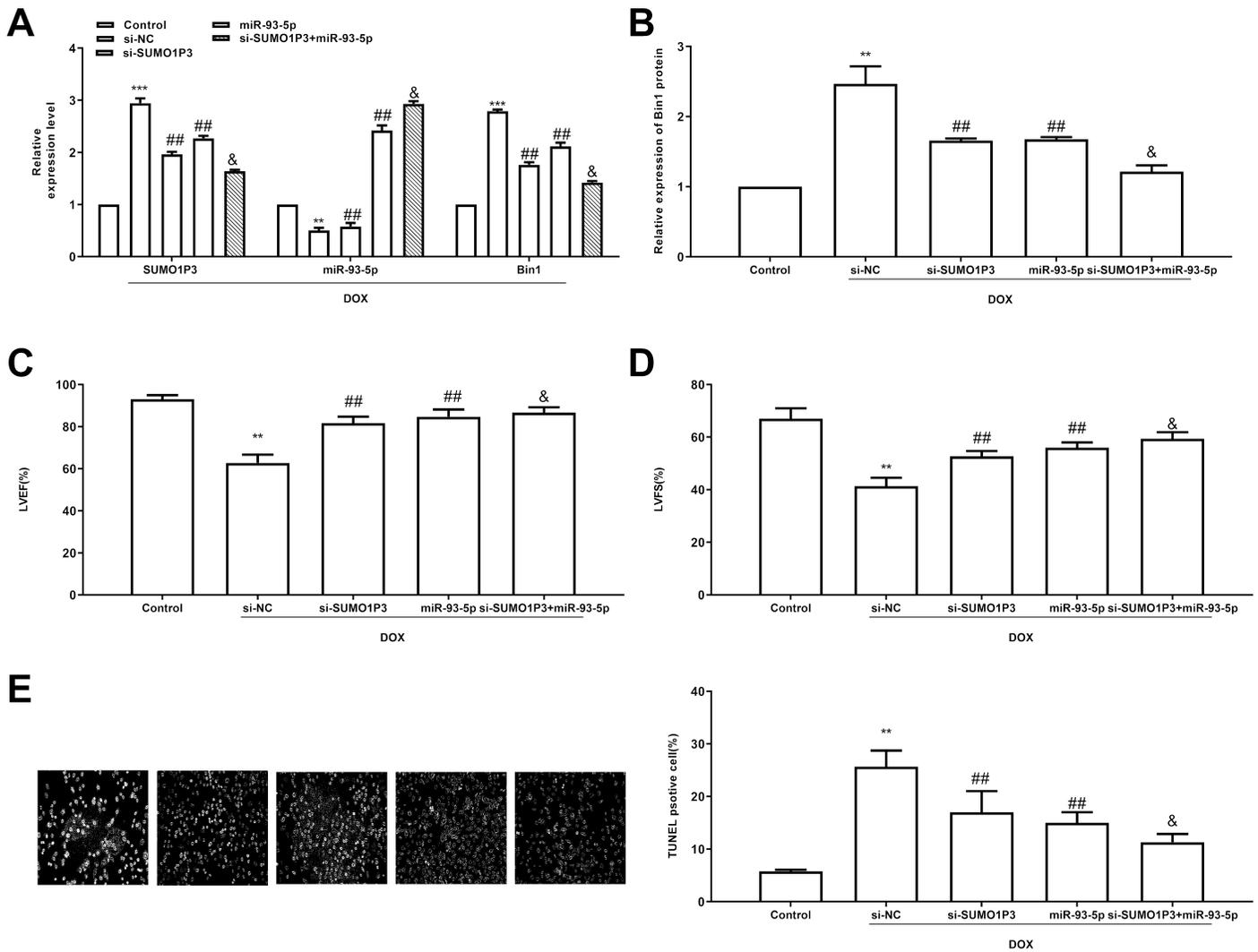


Figure 6

Effect of SUMO1P3 overexpression on myocardial function and apoptosis. (A) Expression levels of SUMO1P3, miR-93-5p, and Bin1 in rats. (B) Protein expression level of Bin1 in rats. (C) Quantitative analysis of LVEF in rats. (D) Quantitative analysis of LVFS in rats. (E) TUNEL assay to detect cardiomyocyte apoptosis. Scale bar = 50 nm. N = 3. ** P < 0.01 vs Control group; ## P < 0.01 vs si-NC group, & P < 0.05 vs si-SUMO1P3 group.