Atorvastatin protects cardiomyocyte from doxorubicin toxicity by modulating YAP/TAZ signaling

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Molecular Biology

Atorvastatin protects cardiomyocyte from doxorubicin toxicity by modulating YAP/TAZ signaling

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This certifies that the Doctoral Dissertation

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Abstract

Atorvastatin protects cardiomyocyte from doxorubicin toxicity by modulating YAP/TAZ signaling

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Yes-Associated Protein (YAP) and Transcriptional Co-activator with PDZ-binding Motif (TAZ) are involved in proliferation and cell survival and are transcriptional co-factors regulated by LATS/MST in the Hippo pathway. In addition, in anticancer drug toxicity studies, YAP and TAZ play an important role in regulating cell apoptosis.

Doxorubicin is an anthracycline anticancer drug, and when it is used in the long term, it causes myocardial toxicity, which leads to heart failure.

Atorvastatin lowers the occurrence of cardiovascular disease by reducing blood cholesterol and the cardiotoxicity of anticancer drugs in cancer patients.

So, we investigated whether YAP and TAZ mediate the protective effect of statin against doxorubicin-induced cardiotoxicity. YAP and its downstream target Park2 decreased when H9c2 cardiomyocytes were treated with doxorubicin. Also, TAZ and its downstream target survivin were decreased. In contrast, atorvastatin treatment restored reduced YAP, TAZ, Park2, and survivin mRNA and protein levels in doxorubicin-induced cardiotoxicity. Furthermore, atorvastatin was decreased expression of cleaved-caspase3 (apoptotic marker) in doxorubicin-induced apoptosis. However, when YAP and TAZ were knocked down by siRNA, atorvastatin could not reduce doxorubicin-induced apoptosis.

Also, Park2 and survivin were not restored by knockdown of YAP and TAZ despite the pre-treatment of atorvastatin in doxorubicin-induced cardiotoxicity. In addition, knockdown of Park2 or survivin attenuated the effect of atorvastatin in inhibiting doxorubicin-induced apoptosis. In chronic or acute doxorubicin-induced cardiomyopathy mice model, oral administration of statin decreased apoptosis of cardiomyocytes.

In conclusion, atorvastatin can prevent doxorubicin-induced cardiotoxicity, and these results showed that atorvastatin exerts antiapoptotic effects by increasing the expression of YAP and TAZ and activating their downstream

target, Park2 and survivin.

Key words: YAP, TAZ, statin, cardiotoxicity, doxorubicin, cardiotoxicity

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I. INTRODUCTION

Heart failure is a high economic and medical burden, which causes high mortality and chronically lowers the quality of life[1]. There are many causes of heart failure, one of which is the side effect of antitumor agents. Recently, as improved survival in cancer patients, the interest in the occurrence of cardiovascular disease among cancer survivors after chemotherapy is

increasing. Particularly, some cancer survivors suffer from heart failure after chemotherapy[2]. Therefore, clinical, basic, and translational research to reduce cardiotoxicity caused by antitumor agents and treatment based on these researches is essential.

Anthracycline antibiotic (doxorubicin) has been used broadly to cure cancer since the 1960s. However, long-term use of doxorubicin in breast cancer patients could cause cardiotoxicity and increase the risk of heart failure[3]. Doxorubicin induces oxidative stress generation, disruption of calcium homeostasis, DNA damage, and cell apoptosis in animal and human pluripotent stem cell-derived cardiomyocytes[4]. However, medical treatments for the cardiotoxicity of doxorubicin are insufficient.

Statin is an HMG-CoA reductase inhibitor that is widely used for dyslipidemia. In addition, statins are very important drugs for managing risk factors and cardiovascular diseases because of their pleiotropic effects, such as neovascularization, immunomodulatory activities, anti-oxidative, anti-inflammatory, and endothelial function improvement. Through these effects, statins can significantly decrease cardiovascular disease and mortality[5].

A recent observational study showed that statin is associated with a 55% lower risk of heart failure in early-stage breast cancer patients treated with anthracyclines. This result supports the protective effect of statin on

anthracycline-related cardiotoxicity[6]. However, several mechanisms have been suggested on how statin reduces the cardiotoxicity of antitumor agents, but studies on molecular biological mechanisms are still insufficient.

Survivin plays an important role in regulating cell survival and proliferation. The physiologic mechanism of survivin in the cardiomyocyte has been explained in a few studies[7]. In a previous study, survivin had a protective effect against doxorubicin-induced cardiomyocytes, and it was a critical protein in the cardiotoxicity model[8,9]. In addition, one suggested mechanism of protection from anthracycline-related cardiotoxicity is that statin suppresses apoptosis of cardiomyocytes by regulating the expression of survivin[10].

Yes-Associated Protein (YAP) and Transcriptional Co-activator with PDZ-binding Motif (TAZ) transcription co-factors are key activators of Hippo signal pathway and are known to control the expression of survivin[7,8]. YAP and TAZ signaling are evolutionarily conserved pathways that control organ size by regulating cell proliferation and apoptosis[11,12]. Small-molecule YAP deletion is a potential new therapeutic strategy for several cancers[13,14]. Also, The YAP and TAZ pathways have been shown to play a crucial role in regulating cardiomyocytes[15].

A recent study showed that YAP loss of function impaired cardiomyocyte proliferation and caused lethal myocardial hypoplasia[16,17]. In addition, deletion

of YAP also reduced cardiomyocyte proliferation as well as infarct increased, and fibrosis worsened after myocardial ischemic injury[17,18]. Furthermore, apoptosis of cardiomyocytes was induced in YAP knockout mice. In contrast, myocardial damage was decreased in YAP-activated mice, and cardiac function was improved despite induction of myocardial ischemic injury[19,20].

Parkin (Park2) plays an important role in cardiovascular diseases such as myocardial ischemia-reperfusion injury, vascular endothelial injury, and diabetic cardiomyopathy[21]. Park2 protein is expressed in various tissues and plays a major role in contributing to protein ubiquitination via stimulating mitophagy[22]. Also, Park2 plays a key role in the mitochondrial apoptosis pathway by regulating the antiapoptotic protein, BCL-2, in the cytoprotective stress response[23]. However, whether Park2 regulates doxorubicin-induced cardiotoxicity is unclear, although the evidence that Park2 is the target protein of YAP is being accumulated[24,25].

According to a recent study, activation of YAP and TAZ, upstream of Park2 and survivin, suppresses apoptosis in damaged cardiomyocytes and regulates the expression of proteins involved in cell survival, which plays a pivotal role in proliferation[26]. These studies have determined that Park2 and survivin play an important role in reducing doxorubicin-induced cardiotoxicity.

However, mechanistic studies of survivin and Park2, which are the target of YAP and TAZ, in cardiomyocyte injury models are insufficient.

Therefore, the purpose of this study is to identify the mechanism of atorvastatin inhibiting doxorubicin-induced cardiotoxicity through Park2 and survivin, which are downstream target proteins of YAP and TAZ.

II. MATERIALS AND METHODS

1. Reagents and antibodies

Doxorubicin was obtained from Tocris. Anti-YAP (sc-101199), anti-Park2 (sc-32282), anti-BCL2 (sc-7382), anti-GAPDH (sc-32233), anti-β-actin (sc-47778) antibodies were obtained from Santa Cruz Biotechnology. Anti-TAZ (#4883), anti-Survivin (#2808), anti-Cleaved-caspase3 (#9661) were purchased from Cell signaling. Atorvastatin were purchased from Sigma Aldrich.

2. Cell culture

The rat heart-derived myoblast cell line, H9c2 cardiomyocytes, was obtained from the American Type Culture Collection (ATCC CRL-1446). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% of penicillin at 37°C in a humidified atmosphere with 5% CO2 and 95% O_2. Subculture were with 1x PBS (Phosphate-Buffered Saline) and 0.25% Trypsin-EDTA within 3~4 days. All experiments were performed using cells between 17 to 30 passage numbers, and all reagents used in the culture were obtained from GIBCO.

After incubation in DMEM containing 10% FBS for 24 h, cells were starved in 1x DMEM containing 0.5% FBS for 18 h. After starvation, cells were pre-

treated with atorvastatin in 0.5% FBS containing DMEM for 1 h prior to doxorubicin treatment for 24 h.

3. Small interfering RNA (siRNA)

When the density of cells reached about 70~80%, Cell were transfected with scrambled RNA or siRNA targeted to YAP, TAZ, Park2 gene using Lipofectamine RNA iMAX (Invitrogen) according to the manufacturer's protocol. Scrambled RNA was purchased from Sigma Aldrich. YAP siRNA (F, 5'-CAA UGA UCA GAC AAC AAC AUU -3'; R, 5'-UGU UGU UGU CUG AUC AUU GUU -3'), TAZ siRNA (F, 5'-GGC CAG AGA UAU UUC CUU AUU -3'; R, 5'-UAA GGA AAU AUC UCU GGC CUU -3'), Park2 siRNA (F, 5'-UUC CAA ACC GGA UGA GUG GUU -3'; R, 5'-CCA CUC AUC CGG UUU GGA AUU -3').

4. Reverse transcription-polymerase chain reaction (RT-PCR)

Cell were harvested and total RNA was extracted using Rneasy mini kit from QIAGEN. The cDNA was synthesized using iScript^{TM} cDNA Synthesis Kit(BIO-RAD), according to manufacturer's instruction. The cDNAs were amplified using AccuPower® PCR PreMix (BIONEER). The sequences of the

primers were as follows: YAP F, 5'-ACC ATA AGA ACA AGA CCA CAT CC-3'; YAP R, 5'-TTC AAT CGC AGC CTC TCC TT-3'; TAZ F, 5'-TTG GGA AGC GGT GGT ACA GG-3'; TAZ R, 5'-ATCAGTCCATTTCCCAGCCTC-3'; Survivin F, 5'- ACC CTA TAG AGG AGC ATA GGA AG -3'; Survivin R, 5'-GGC TCT TTG TTT GTC CAG TTT C-3'; GAPDH F, 5'-AAT GCA TCC TGC ACC ACC AAC TGC-3'; GAPDH R, 5'-GGA GGC CAT GTA GGC CAT GAG GTC-3'; Park2 F, 5'-GAC CAG CTG CGA GTG ATT T-3'; Park2 R, TCC TCT GTG GTC TCT GTA CTA TG-3'. PCR products were separated by electrophoresis in a 1~2% agarose gel containing Gel-red (Biotium).

5. Quantitative real-time Polymerase Chain Reaction (qRT-PCR)

Real-time qRT-PCR analysis was performed using a SYBR® Premix EX TaqTM (TaKaRa) and StepOnePlus Real-Time PCR System (ThermoFisher) in order to determine the Quantitative mRNA expression levels of the target genes. Simply, cDNA was pre-incubated at 95℃ for 30sec, followed by 40 cycles of denaturation at 95℃ for 5sec, annealing at 64 ℃ for 34sec.

6. Confocal immunofluorescence microscopy

Immunofluorescence Confocal microscopy was performed as previously studies. Simply, H9c2 cardiomycyte cells cultured on Lab-Tek chamber slides

(Nalgene Nunc, USA) were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. After blocking with PBS containing 10% horse serum and 1% bovine serum albumin, the slides were incubated with anti-YAP, anti-TAZ, anti-survivin, anti-Park2 antibody and mounted with containing DAPI. Immune response signals were visualized with a confocal laser scanning microscope LSM700 (Carl Zeiss, Germany).

7. Immunoblot analysis

H9c2 cardiomyocyte cells and mice heart tissue proteins were extracted using RIPA (50 mM Tris-HCl pH7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA) lysis buffer, containing protease and phosphatase inhibitors at 4℃ for 15 min. After centrifuged at 4℃ for 40 min or 1hr at 13,000 g the supernatants were collected. The cell lysate (15 μg-35 μg) of each sample were fractionated by SDS polyacrylamide gels, and then transferred to PVDF membranes, which were blocked with 5% skim-milk or 5% BSA in TBS-T with 0.1% Ttween-20 for 1hr at RT (room temperature). After, the membranes were immunoblotted with primary antibody at 4℃ overnight. Then cells were incubated with HRP conjugated secondary antibodies. Antigen-antibody complexes were assessed by amplified Chemiluminescence.

8. TUNEL assay and Immunohistochemical staining

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was executed with a commercially available kit, DeadEnd™ Fluorometric TUNEL System (Promega) according to the manufacturer's instructions. Briefly, Formalin-fixed, paraffin-embedded mice heart tissue sections (4μm thick) were deparaffinized, rehydrated, and washed with 1x PBS. They were blocked with PBS containing 2.5% horse serum for 1hr at room temperature and incubated and primary antibodies applied overnight at 4°C. After, slides were incubated with FITC-488, Rhodamine Red-X as secondary antibody, diluted 1:200 in PBS, for 1hr in dark room. After washing in PBS, the slides were mounted with VECTASHIELD® Antifade Mounting Media With DAPI (VECTOR). The immunoreactive signals were visualized by confocal microscope LSM700 (Carl Zeiss, Germany).

9. Animal studies

Mice were maintained according to standard approved Institutional Animal Care Use Committee protocols at Yonsei University College of Medicine (2021-0143). The animals were maintained in pathogen-free cages and light, as well as temperature-controlled rooms provided with rodent chows and sterile water. C57BL6 mice of seven-weeks-old were obtained Orient-Bio, and

randomly divided in to three groups (saline group, doxorubicin group and atorvastatin + doxorubicin group). After 6 weeks, cardiac MRI was performed before sacrificed and heart tissues were obtained. And for protein isolation, hearts were instantly transferred to a nitrogen tank and deep-freezer at 70℃.

10. Tissue staining (H&E, Masson's Trichrome)

Mice hearts were observed by perfused with saline and fixed in 4% paraformaldehyde for 24h at 4℃. Then, they were embedded in paraffin and prepared in 4μm cross sections. Mice hearts were stained with conventional hematoxylin and eosin (H&E) and Masson's trichrome for analysis of histology and fibrosis.

11. Transmission electron microscope

Mice hearts were fixed for 12hr in 2% Glutaraldehyde – Paraformaldehyde in 0.1M phosphate buffer (pH 7.4) and washing in 0.1M phosphate buffer. They were fixed with 1% OsO_4 dissolved in 0.1M PB for 2hr and dehydrated in gradual series (50 ~ 100%) of ethanol and infiltrated with propylene oxide. Sections slide of about 200~250nm thick section were at first cut and stained with toluidine blue (sigma, T3260) for optical microscope. The 70nm thin section was double stained with 6% uranyl acetate (EMS, 22400 for 20mins)

and lead citrate (fisher, for 10mins) for contrast staining. Their sections were cut by LEICA EM UC-7(Leica Microsystems, Austria) with a diamond knife(Diatome) and transferred on copper and nickel grids. All thin sections were observed with a transmission electron microscope (JEM-1011, JEOL, Japan) at an acceleration voltage of 80 kV.

12. Statistical analysis

Three or five experiments were performed for in vitro studies. All results are indicated as means ± SEM. Significance was determined using one-way ANOVA with Tukey or tow-tailed Student's *t*-test analysis of variance, as appropriate, for multiple comparison by Prism 5 (GraphPad Software, USA). Values of $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ were defined as statistically significant.

III. RESULTS

1. Doxorubicin decreased the expression of YAP and TAZ in the H9c2 cardiomyocyte cell line in a time-dependent manner

To investigate the role of YAP and TAZ in doxorubicin-induced cardiotoxicity, cardiomyocytes were treated with doxorubicin in a time-dependent manner. The H9c2 cells were treated with doxorubicin time dependently, and the expression of YAP and TAZ was evaluated by western blot analysis and RT-PCR. The Figure 1A showed that doxorubicin decreased the protein expression of YAP and TAZ in the H9c2 cells in a time-dependent manner. Similarly, the effect on mRNA expression was evaluated through RT-PCR (Figure 1B). These data suggest that doxorubicin significantly reduces the expression of YAP and TAZ in H9c2 cardiomyocytes.

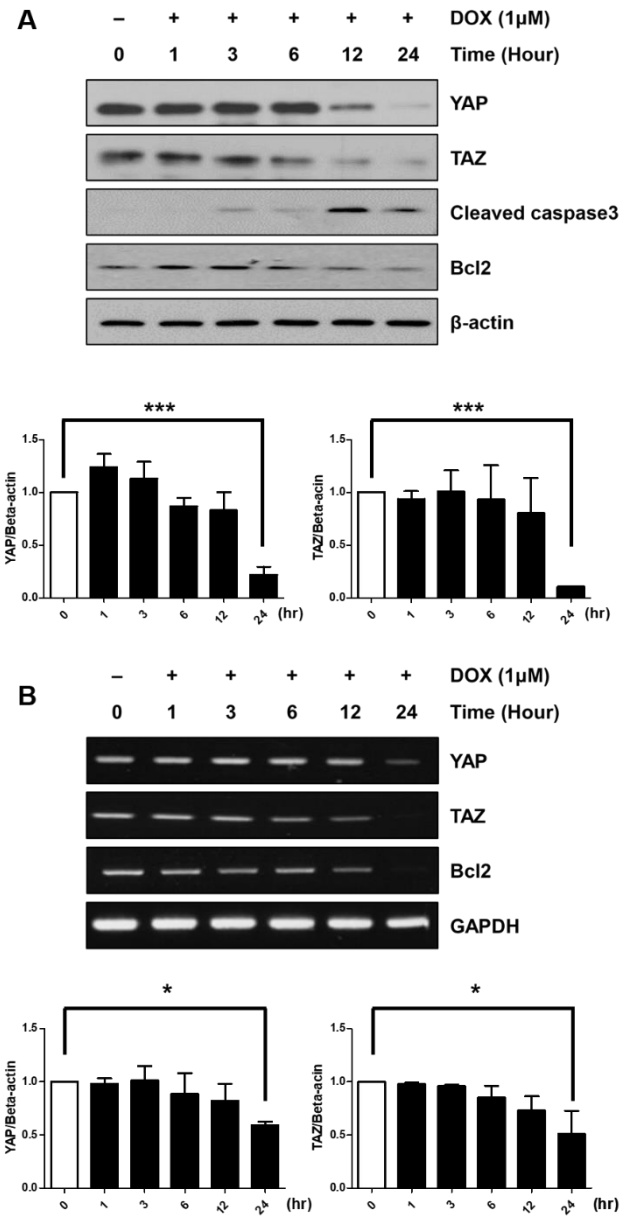


Figure 1. A-B legend (following pages)

Figure 1. Effects of doxorubicin on YAP and TAZ transcription level in H9c2 cardiomyocyte cell line.

(A-B) The H9c2 cardiomyocytes were treated with doxorubicin (1uM) in a time-dependent manner. Expression of YAP and TAZ was determined using Western blot analysis. Relative levels of YAP versus β-actin, and TAZ versus β-actin in each sample as determined by blot densitometry. (B) Total RNA was analyzed by RT-PCR (28 cycles) using primers specific to YAP, TAZ and GAPDH gene, which show that equal amounts of sample were loaded. Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

2. Atorvastatin restored decreased expression of YAP and TAZ at translation and transcription levels in doxorubicin-induced cardiotoxicity.

We hypothesized that atorvastatin could rescue decreased YAP and TAZ expression in doxorubicin-induced cardiotoxicity. To investigate whether YAP and TAZ expression was affected by atorvastatin, immunoblot analysis and qRT-PCR were performed. Interestingly, atorvastatin recovered decreased YAP/TAZ protein (Figure 2A) and mRNA (Figure 2B) levels on the doxorubicin-treated H9c2 cardiomyocytes. These results showed that YAP and TAZ expression was transcriptionally regulated in doxorubicin-induced cardiotoxicity. Also we investigated the protein expression of cleaved-caspase3 (active form) and Bcl2, which were well-recognized indicators of apoptosis. Atorvastatin inhibited the doxorubicin effect that increased cleaved-caspase3 and decreased Bcl2.

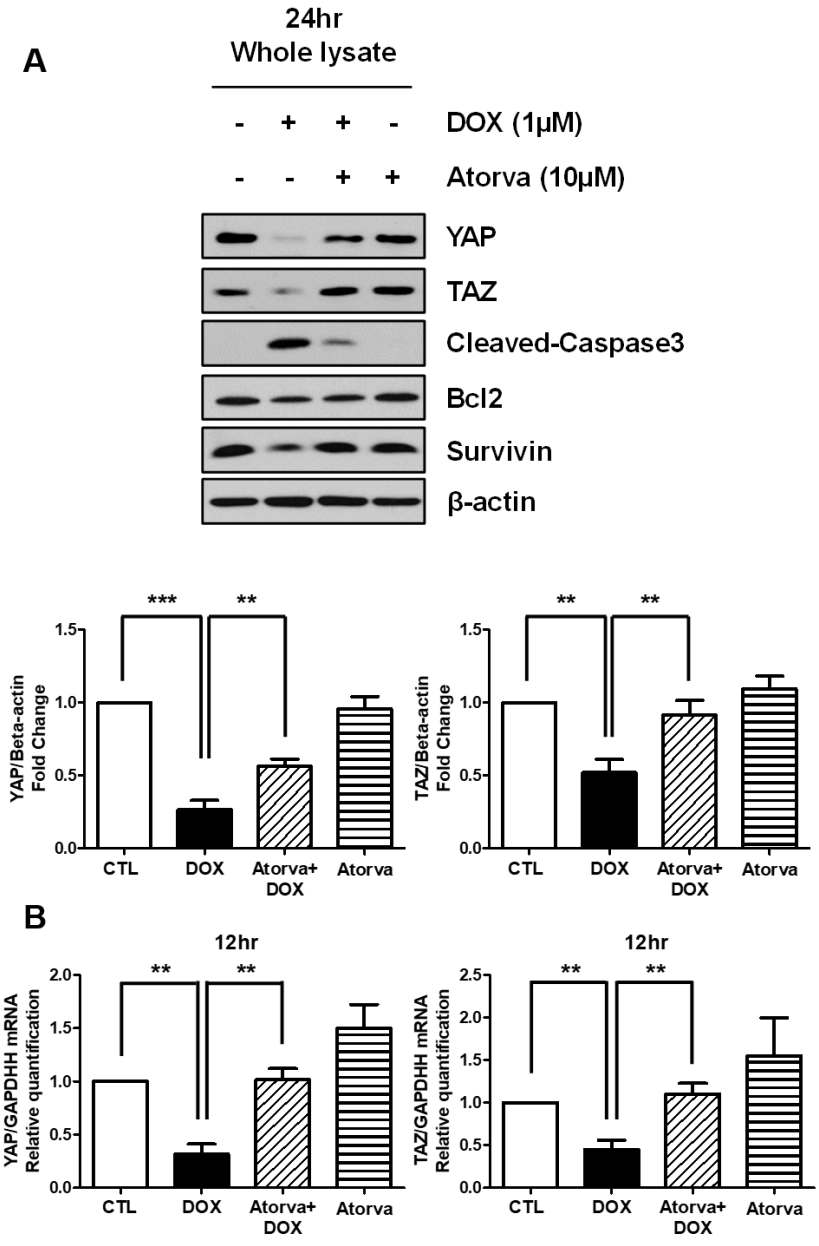


Figure 2. A-B legend (following pages)

Figure 2. Effect of atorvastatin on doxorubicin-induced decreased expression of YAP and TAZ in H9c2 cardiomyocyte cell line

(A-B) The H9c2 cardiomyocytes were treated with or without 10μM atorvastatin for 60 min and then were exposed to doxorubicin for 24 hr. (A) The same amount of protein was separated by SDS-PAGE gel, and immunoblot analysis was performed using anti-YAP and TAZ, anti-Cleaved-caspase3, anti-Bcl2 and anti-β-actin antibody. (B) Total RNA was analyzed by qRT-PCR using primers specific to YAP and TAZ gene. qRT-PCR was performed on cells treated with doxorubicin for 12 hr. Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

3. Immunofluorescence demonstrated that atorvastatin restored doxorubicin-induced decreased expression of YAP and TAZ.

In previous data, we confirmed that atorvastatin restored expression level of YAP and TAZ in doxorubicin-induced cardiotoxicity. The experiment, using immunofluorescence, was conducted to confirm that the decreased expression of YAP and TAZ in doxorubicin-induced cardiotoxicity could be restored by atorvastatin. YAP and TAZ expression were significantly reduced by doxorubicin and restored by atorvastatin (Figure 3A and B). These results suggested that atorvastatin may play a role in preventing the decrease of YAP/TAZ by doxorubicin.

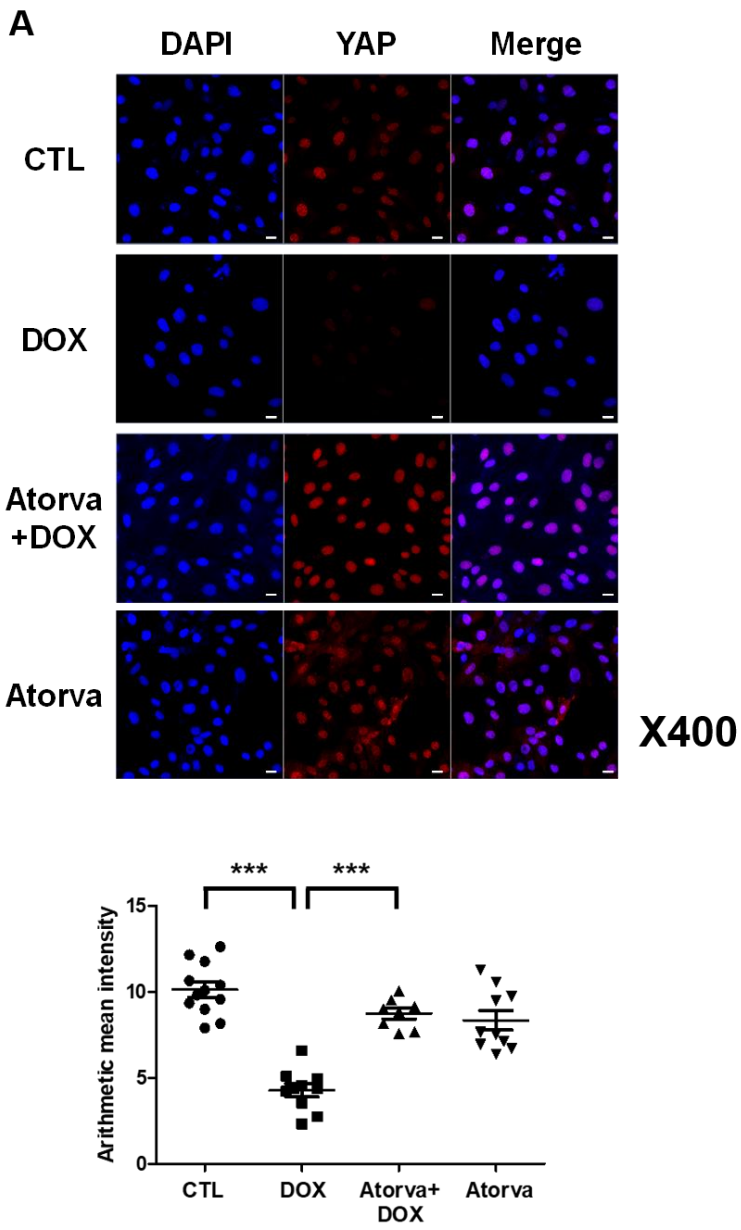


Figure 3. A legend (following pages)

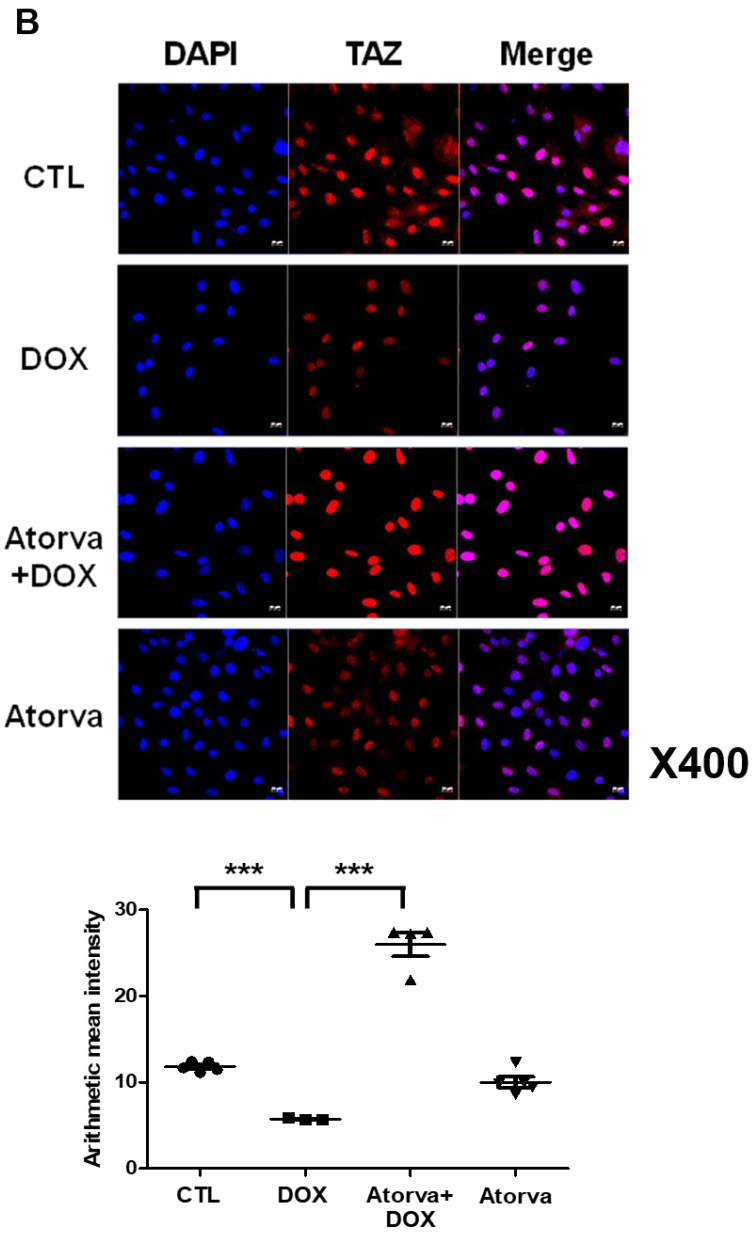


Figure 3. B legend (following pages)

Figure 3. Immunofluorescence showed that atorvastatin restored decreased expression of YAP and TAZ in doxorubicin-induced cardiotoxicity.

(A-D) The H9c2 cardiomyocytes were exposed with or without pretreatment with 10μM of atorvastatin for 60 minutes in addition to doxorubicin treatment for 24 hours. (A) YAP protein was also observed with confocal microscopy using primary anti-YAP antibody and Alexa Fluor™ 594-conjugated secondary antibody (400x). (B) TAZ protein was observed with confocal microscopy using Rhodamine-conjugated secondary antibody (400x). Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

4. Doxorubicin decreased the expression of Park2 in the H9c2 cardiomyocyte cell line in a time-dependent manner.

According to our study, cardiomyocytes with YAP and TAZ activation have a lower apoptosis and higher expression of anti-apoptotic genes such as survivin. Park2 is one of YAP regulated gene and it is known to be involved in mitochondrial biogenesis [27]. The H9c2 cells were treated with doxorubicin for time-dependent manner, and the effect on the expression of Park2 was evaluated by Western blot analysis and RT-PCR. (Figure 4 A and B). It shows a time-dependent decrease in Park2 protein levels when treated with 1μM of doxorubicin for variable times (Figure 4A). Also, changes in Park2 mRNA levels were decreased under the same conditions (Figure 4B). These data suggested that doxorubicin reduced the translationally and transcriptional expression of Park2 in H9c2 cardiomyocytes.

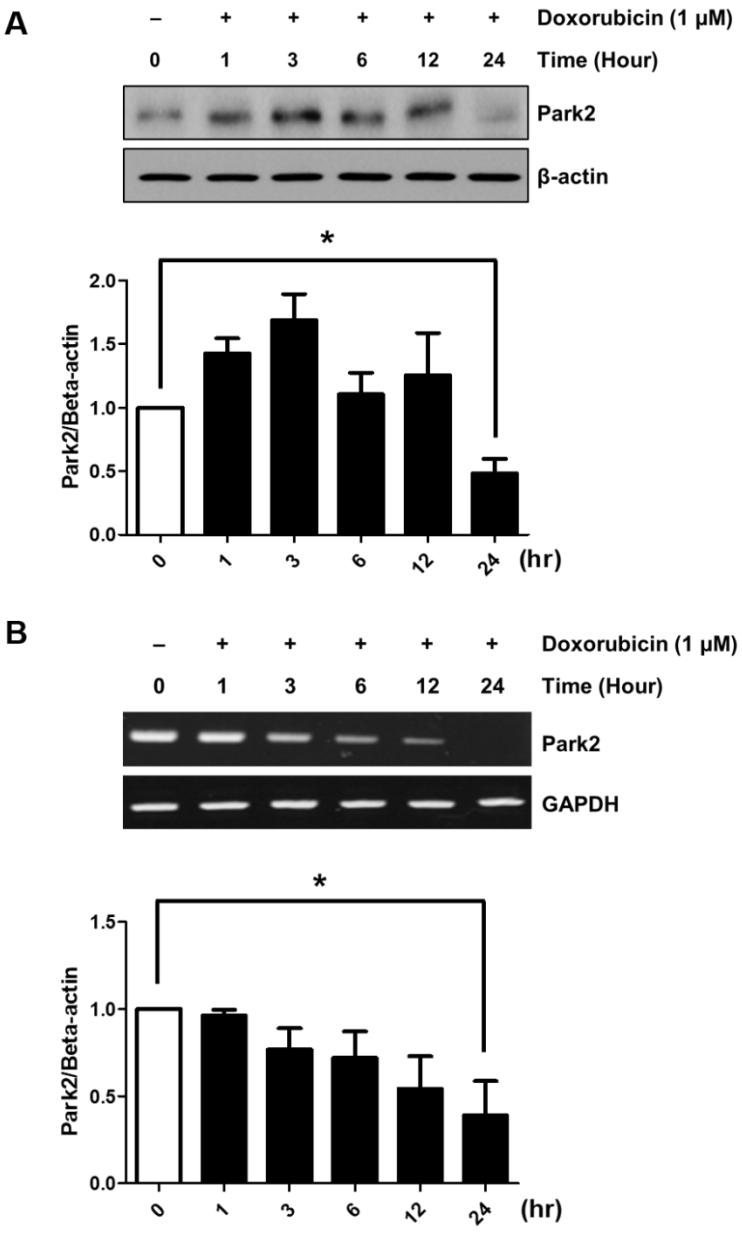


Figure 4. A-B legend (following pages)

Figure 4. Effects of Park2 expression on doxorubicin-induced H9c2 cardiomyocyte cell line.

(A-B) The H9c2 cardiomyocytes were treated with doxorubicin (1μM) in a time-dependent manner. (A) Expression of Park2 was observed through Western blot analysis. Relative levels of Park2 versus β-actin in each sample was determined by blot densitometry. (B) Total RNA was analyzed by RT-PCR (32 cycles) using specific primers as Park2 and GAPDH gene. Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

5. Atorvastatin restored doxorubicin-induced decreased expression of Park2 at translation and transcription level.

We examined whether atorvastatin attenuates decrease of Park2 in doxorubicin-induced cardiotoxicity. H9c2 cardiomyocytes were pretreated with 10μM of atorvastatin for 1hr prior to doxorubicin treatment (Figure 5A and B). Park2 protein and mRNA levels were decreased in H9c2 cardiomyocytes treated with doxorubicin, and these decreases were inhibited by atorvastatin pretreatment. The result shows that atorvastatin restored decrease of Park2 expression on the doxorubicin-induced cardiotoxicity.

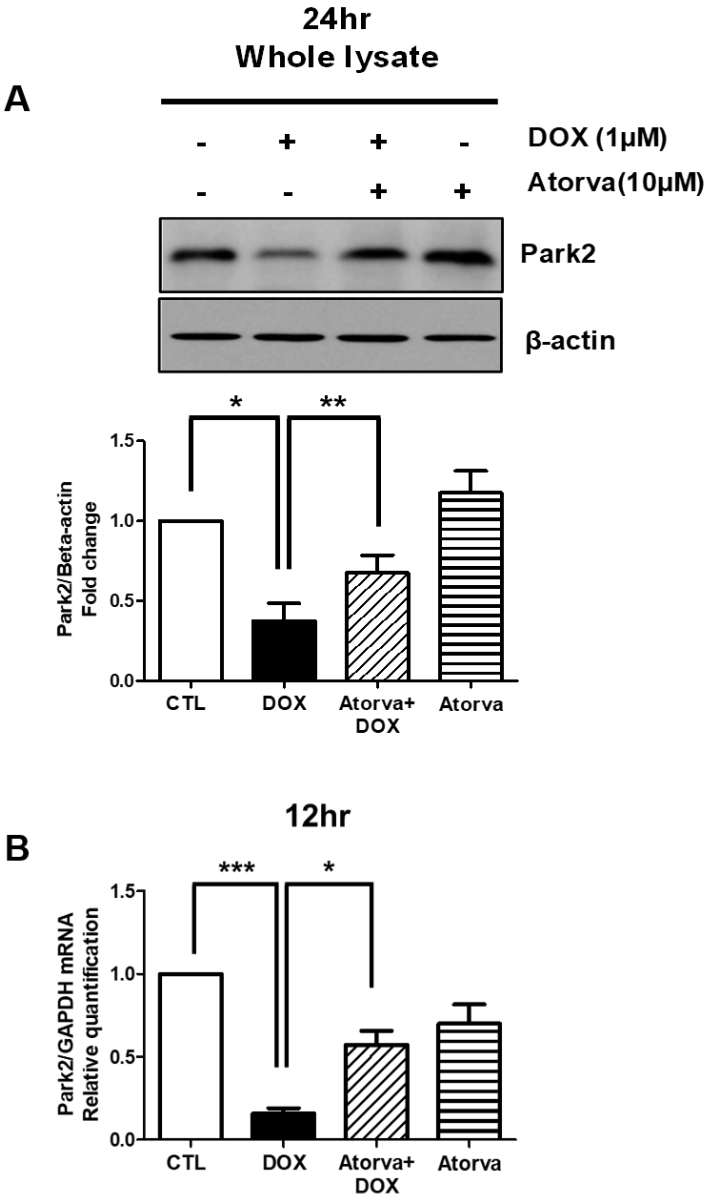


Figure 5. A-B legend (following pages)

Figure 5. Atorvastatin restored decreased Park2 expression level by doxorubicin.

(A) The H9c2 cardiomyocytes were pretreated with atorvastatin (10μM) for 1hr prior to doxorubicin (1μM) treatment for 24hr. Park2 protein level was evaluated by Western blotting. (B) Cells were pretreated with or without atorvastatin (10μM) prior to doxorubicin (1μM) for 12hr. mRNA expression level was measured by qRT-PCR. Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

6. Immunofluorescence demonstrated that atorvastatin restored doxorubicin-induced decreased expression of Park2.

In previous data, we confirmed that the reduction of Park2 protein and mRNA level was restored by atorvastatin in doxorubicin-induced cardiotoxicity. Decreased Park2 expression was visualized by fluorescence staining to determine whether doxorubicin was restored by atorvastatin. The result suggests that expression of Park2 was restored by atorvastatin pretreatment in H9c2 cardiomyocytes (Figure 6A).

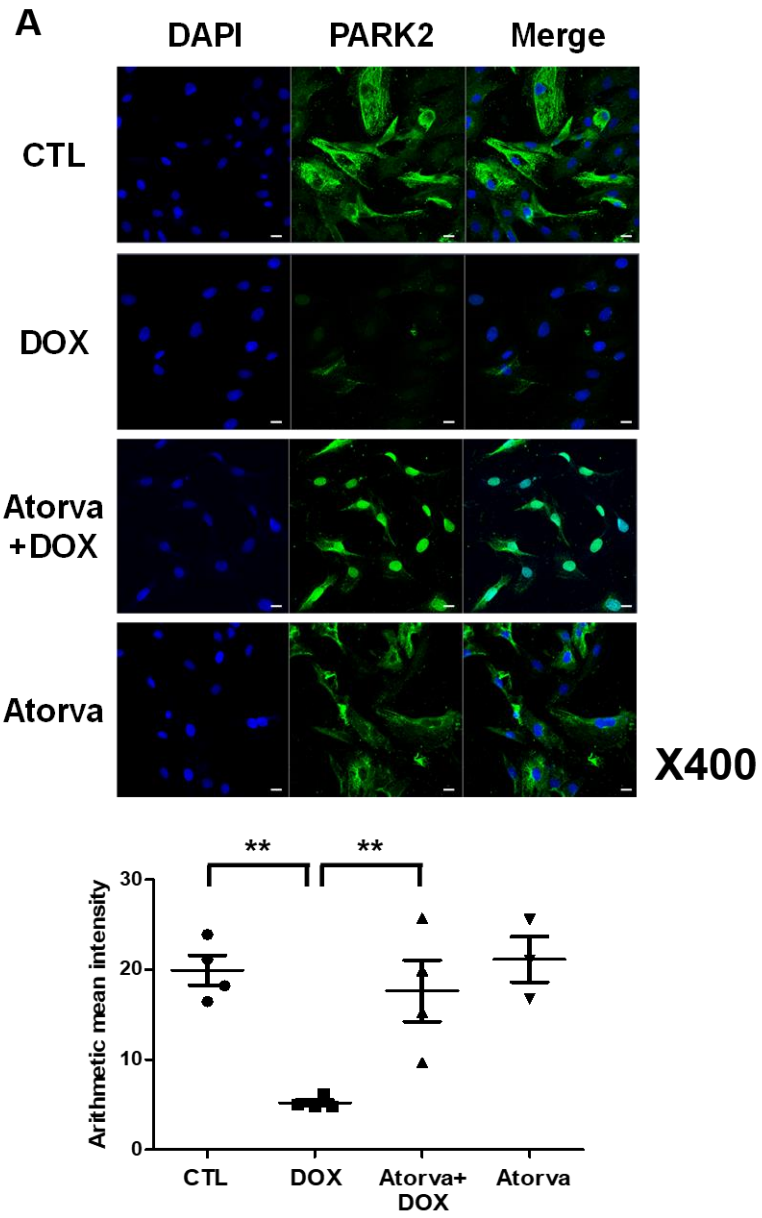


Figure 6. A legend (following pages)

Figure 6. Immunofluorescence showed that atorvastatin restored decreased expression of Park2 in doxorubicin-induced cardiotoxicity.

The H9c2 cardiomyocytes were exposed with or without pretreatment with atorvastatin (10μM) for 1hr in addition to doxorubicin (1μM) treatment for 24hr. (A) Park2 protein was also observed with confocal microscopy using primary anti-Park2 antibody and Alexa Fluor™ 488-conjugated secondary antibody (400x). Data are shown as the means ±SEM (n=3 or n=5). **P<0.01.

7. Doxorubicin decreased the expression of survivin in H9c2 cardiomyocyte cell line in a time-dependent manner.

As a downstream target of the YAP/TAZ signal, we investigated the change of survivin in doxorubicin-treated cardiac cells. According to previous studies, survivin plays an important role of survival in H9c2 cardiomyocyte cell line. It was confirmed that survivin protein levels were decreased by doxorubicin in a time-dependent manner (Figure 7A). And the RT-PCR showed that the mRNA of survivin was decreased time-dependently by doxorubicin treatment (Figure 7B).

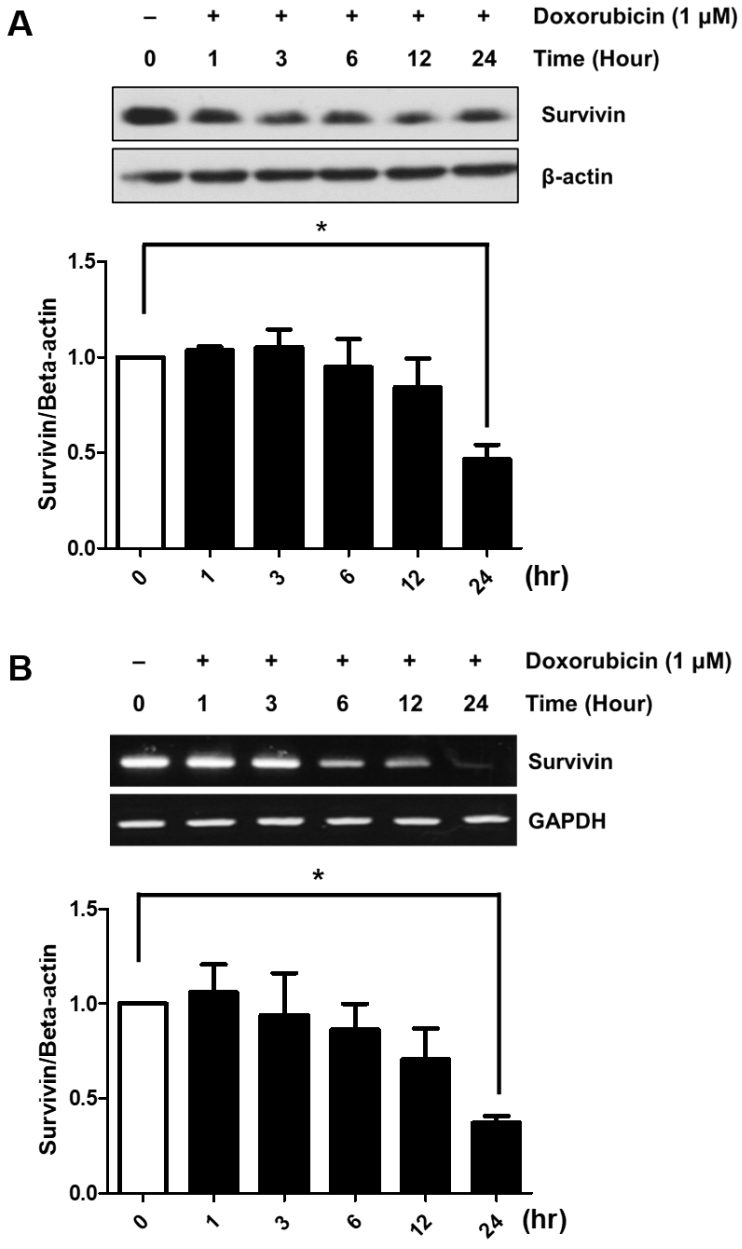


Figure 7. A-B legend (following pages)

Figure 7. Doxorubicin decreased the expression of survivin in H9c2 cardiomyocyte cells in a time-dependent manner.

H9c2 cardiomyocytes were exposed to doxorubicin (1μM) for the indicated time periods. (A) The protein levels of survivin were determined using western blot analysis. (B) Using RT-PCR, the mRNA levels of survivin over time was analyzed in doxorubicin-treated H9c2 cells. Total RNA was analyzed by RT-PCR (32 cycles) using specific primers to survivin and GAPDH gene, which bands show that equal amounts of sample were loaded. Data are shown as the means ±SEM (n=3 or n=5). *P<0.05.

8. Atorvastatin recovered doxorubicin-induced decreased expression of survivin at translation and transcription levels.

Western blot and qRT-PCR were performed to determine whether the survivin reduction caused by doxorubicin was attenuated by atorvastatin. Western blot and qRT-PCR revealed that survivin is significantly decreased in doxorubicin-treated cardiomyocytes compared to controls group and atorvastatin group (Figure. 8A and B). When atorvastatin was pretreated, decreased of survivin by doxorubicin was not shown.

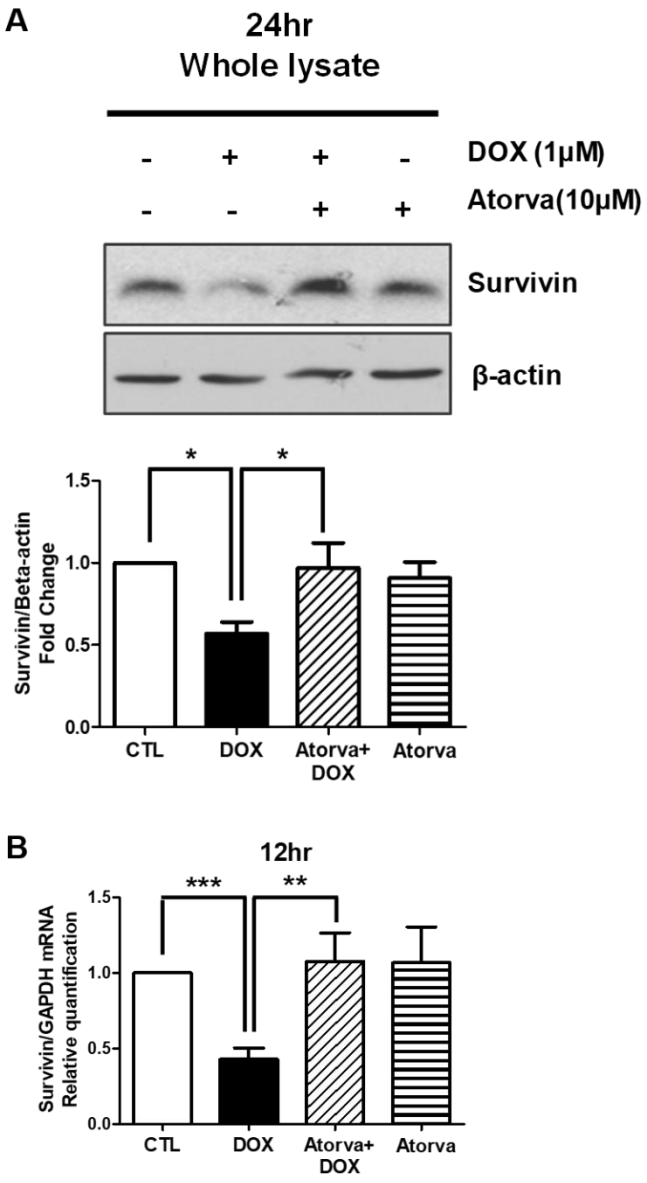


Figure 8. A-B legend (following pages)

Figure 8. Atorvastatin restored decreased survivin transcription level induced by doxorubicin

(A) The H9c2 cardiomyocytes were pretreated with atorvastatin (10μM) for 1hr prior to doxorubicin (1μM) treatment for 24hr. Survivin protein level was evaluated by Western blotting. (B) Cells were pretreated with or without atorvastatin (10μM) prior to doxorubicin (1μM) for 12hr. mRNA expression level was measured by qRT-PCR. Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

9. YAP knockdown inhibited the recovery of Park2 expression by atorvastatin in doxorubicin-induced cardiotoxicity

To investigate whether YAP regulates Park2 transcriptional expression, H9c2 cardiomyocyte was transiently transfected with YAP targeted siRNA. And, immunoblot analysis and qRT-PCR were performed for demonstrating the change of Park2 expression. Surprisingly, knockdown of YAP significantly reduced expression of Park2 recovered by atorvastatin in doxorubicin-induced cardiotoxicity (Figure. 9A and B). The ability of atorvastatin to inhibit doxorubicin-mediated cleaved-caspase 3 activation was completely perturbed by YAP knockdown.

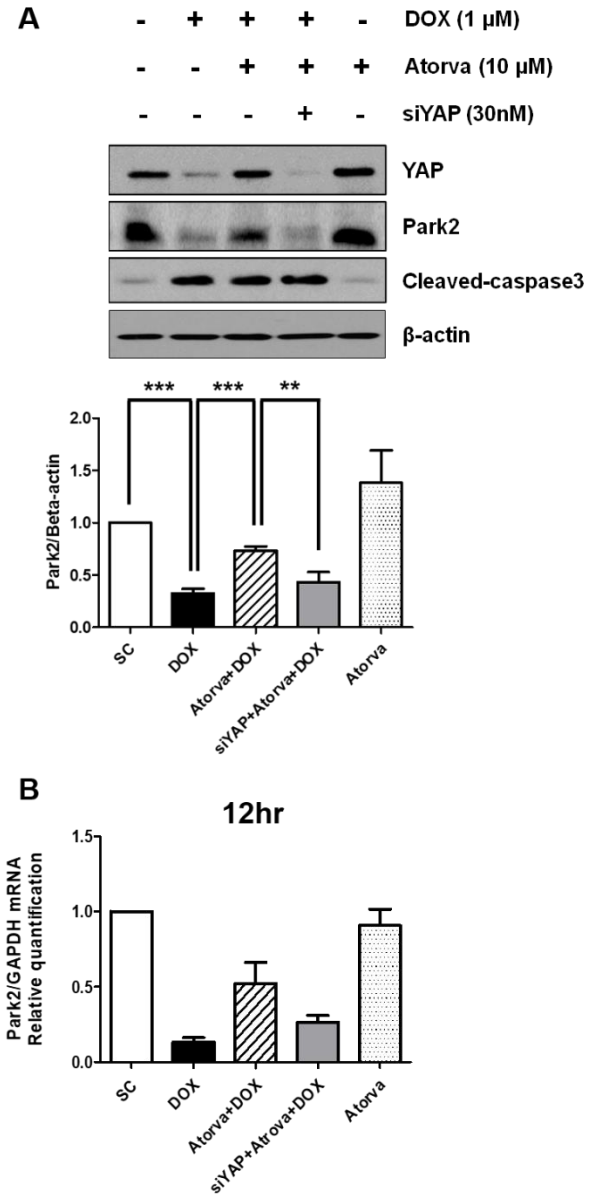


Figure 9. A-B legend (following pages)

Figure 9. YAP knockdown reduced Park2 expression in doxorubicin-induced cardiotoxicity.

(A-B) The H9c2 cardiomyocytes were transfected with YAP siRNA. And, the H9c2 cardiomyocytes were pretreated with atorvastatin (10μM) for 1hr prior to doxorubicin (1μM) treatment for 24hr. After siRNA transfection, whole lysates were immunoblotted and mRNA was analyzed by qRT-PCR for Park2 expression. (A) Equal amounts of protein were separated by SDS-PAGE gel, and immunoblot analysis was performed using anti-Park2 antibody and (B) total RNA was analyzed by qRT-PCR (40 cycles). Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

10. TAZ knockdown inhibited the recovery of survivin expression by atorvastatin in doxorubicin-induced cardiotoxicity

Survivin was a critical transcription factor in regulation of cell survival in doxorubicin-induced cardiac injury model. Also, in cancer cell, YAP/TAZ signaling is another important transcription factor for regulating survivin transcription [28]. So we hypothesized that TAZ could be another transcriptional regulator for survivin expression in cardiomyocytes. To investigate whether TAZ regulates survivin transcriptional expression H9c2 cardiomyocyte were transiently transfected with TAZ targeted siRNA. Both qRT-PCR and Western blot analysis results indicate that TAZ knockdown significantly decreases survivin expression (Fig. 10A and B). Atorvastatin attenuates decrease of survivin via doxorubicin but was completely perturbed due to TAZ knockdown.

Also, we investigated the protein expression of cleaved-caspase3 (active form), which was a well-recognized indicator of apoptosis. As shown in Figure 10 A, the protein expression of cleaved-caspase3 (active form) were significantly increased in TAZ silenced H9c2 cardiomyocytes. These results suggest that TAZ deficiency could induce apoptosis in H9c2 cardiomyocytes.

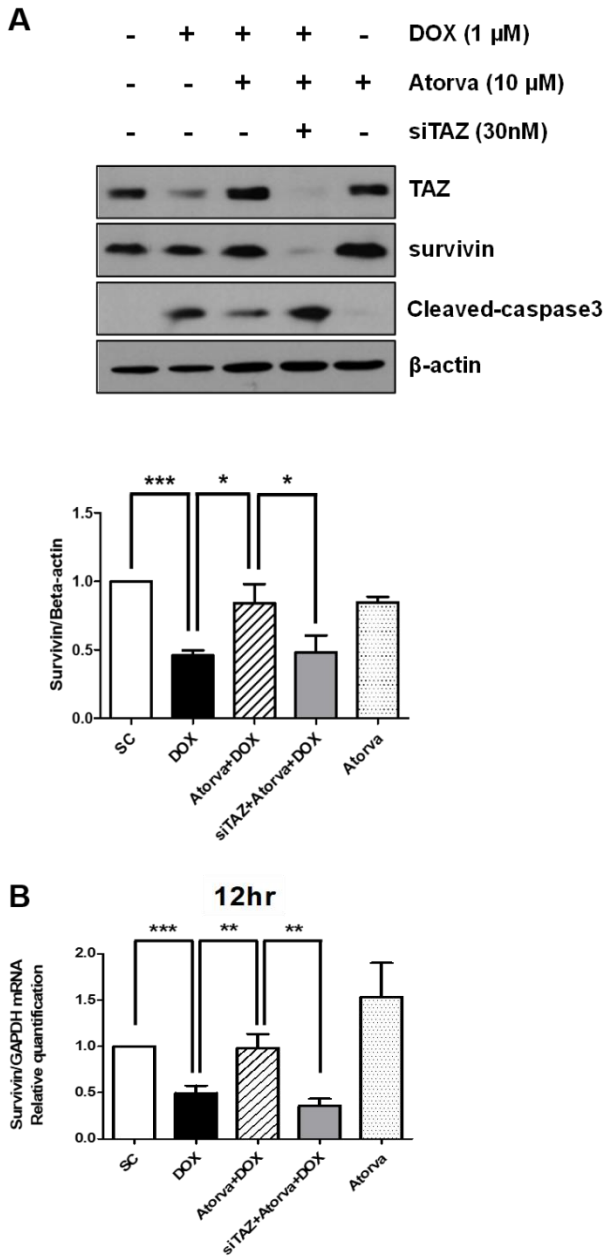


Figure 10. A-B legend (following pages)

Figure 10. TAZ knockdown reduced Survivin expression in doxorubicin-induced cardiotoxicity.

(A-B) The H9c2 cardiomyocytes were transfected with TAZ siRNA. And, the H9c2 cardiomyocytes were pretreated with atorvastatin (10μM) for 1hr prior to doxorubicin (1μM) treatment for 24hr. After siRNA transfection, whole lysates were immunoblotted and mRNA was analyzed by qRT-PCR for survivin expression. (A) Equal amounts of protein were separated by SDS-PAGE gel, and immunoblot analysis was performed using anti-survivin and anti-cleaved-caspase3 antibodies and (B) total RNA was analyzed by qRT-PCR (40 cycles). Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

11. Park2 knockdown inhibited the recovery of cell apoptosis by atorvastatin in doxorubicin-induced cardiotoxicity

The role of Park2 in atorvastatin-mediated H9c2 cell protection was investigated by silencing of Park2. H9c2 cardiomyocyte were transfected with the Park2 targeted siRNA to reduce its expression. Park2 knockdown also increased cleaved-caspase3 expression. The effect of atorvastatin to inhibit doxorubicin-mediated cleaved-caspase 3 activation was completely perturbed by Park2 knockdown (Figure 11A). These findings suggest that Park2 plays a role in the cell protection of atorvastatin in the doxorubicin induced cardiotoxicity.

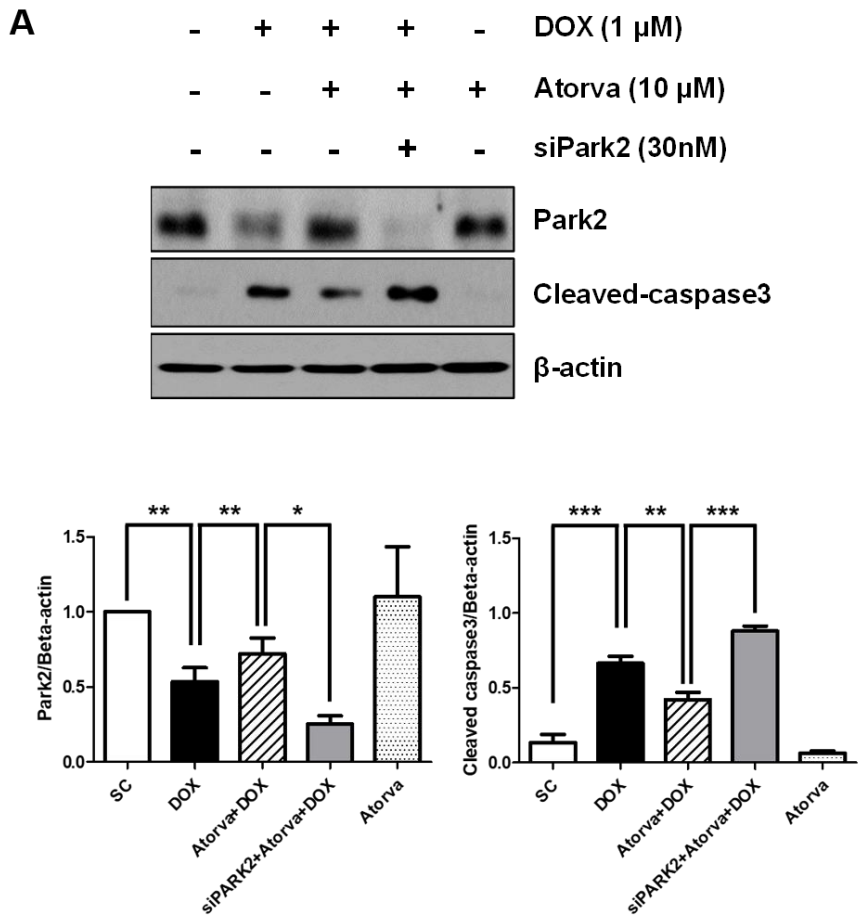


Figure 11. A legend (following pages)

Figure 11. Park2 knockdown led to activation of cleaved-caspase3.

A day after transfection with both scrambled siRNA or Park2 siRNA, H9c2 cardiomyocytes were untreated or pretreated with atorvastatin(10uM) for 1hr and then exposed to doxorubicin (1μM) for 24hr (A). Cell lysates were immunoblotted with antibodies against Park2, cleaved-caspase-3(active form) and β-actin. Data are shown as the means ±SEM (n=3 or n=5). *P<0.05, **P<0.01, ***P<0.001.

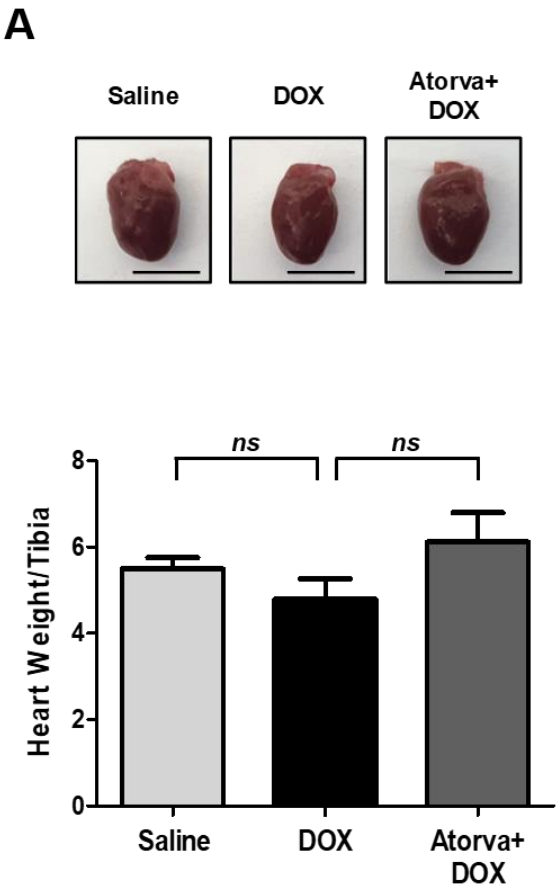
12. Atorvastatin attenuated myocardial dysfunction in doxorubicin-induced cardiac injury mice model.

(Figure 12A - D) To investigate the effect of atorvastatin on doxorubicin-induced cardiac injury model, 8-weeks-old C57BL/6 mice were pretreated with 10mg/kg of atorvastatin or normal saline once a day by oral gavage for 6weeks. C57BL/6 mice were treated with 5mg/kg of doxorubicin by intraperitoneal injection, once a week for 5 weeks. Mice heart tissues were isolated and paraffin-embedded for Haematoxylin & Eosin staining and Masson's trichrome staining. (Figure 12B)

The results of H&E stain showed multifocal areas of patchy and scattered cardiomyocyte with vacuolation in doxorubicin group, which was significantly recovered in atorvastatin + doxorubicin group. The Masson's trichrome staining exhibited that fibrosis areas, proved with blue staining areas, were increased in doxorubicin treatment group. But they were recovered in atorvastatin + doxorubicin group.

Moreover, TEM (Transmission electron microscope) showed less myofibrillar disarray and mitochondrial destruction in atorvastatin + doxorubicin group, but they were deteriorated in doxorubicin group (Figure 12C). Furthermore, cardiac protective effects of statin were confirmed by measuring LVEF through MRI imaging in doxorubicin-induced cardiac

injury model. The LVEF was reduced in doxorubicin group but, it was also significantly recovered in atorvastatin + doxorubicin group (Figure 12D). (Saline vs. DOX mice: LVEF, 67.11 ± 1.59 vs. 47.75 ± 4.94%, **P<0.01, DOX vs. Atorva + DOX mice: LVEF, 47.75± 4.94 vs. 57.01 ± 5.67%, *P<0.05 Figure. 12D).



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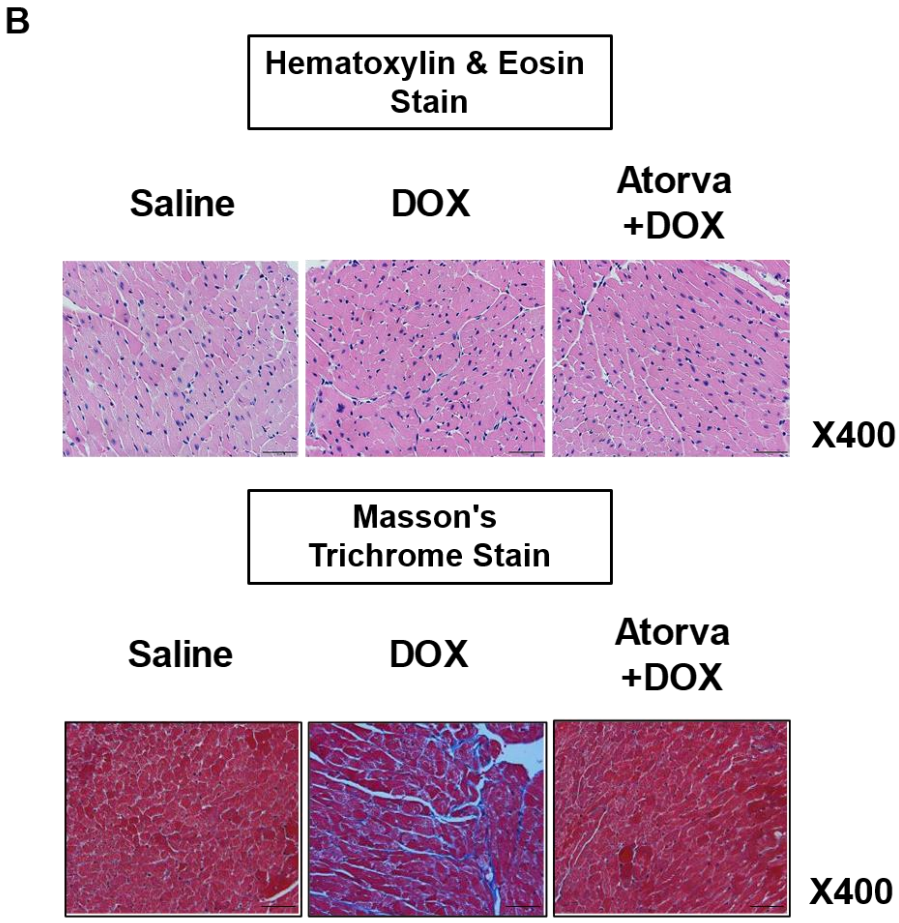

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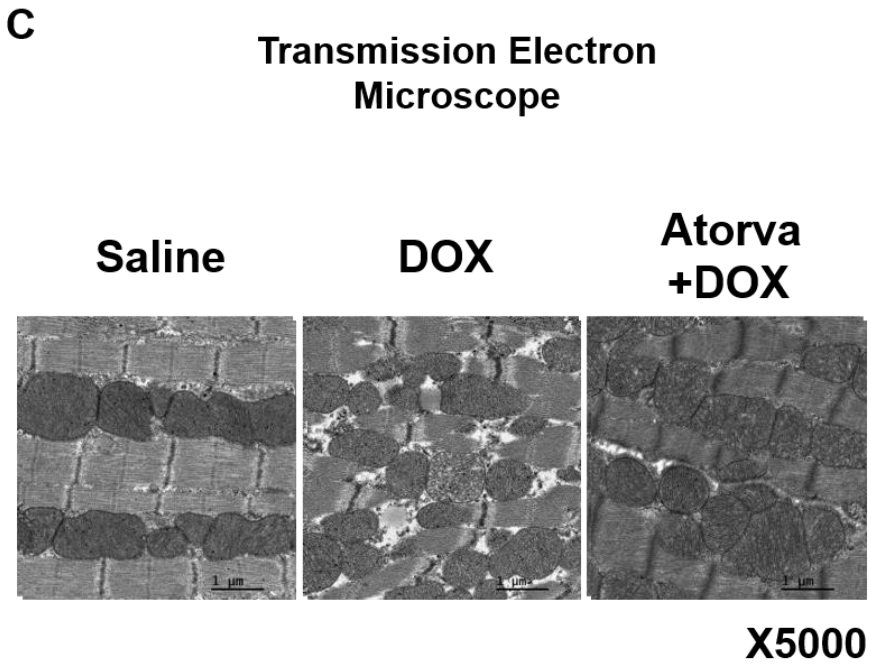

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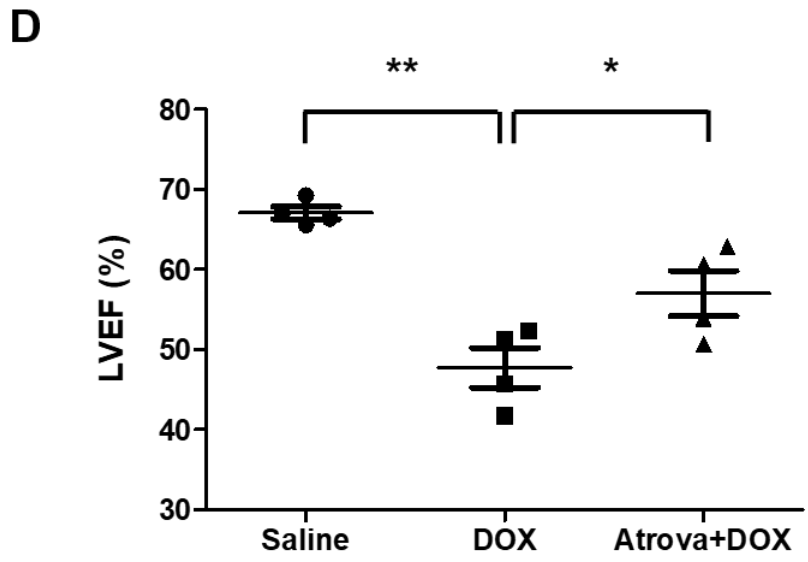


Figure 12. D legend (following pages)

Figure 12. Atorvastatin recovered doxorubicin-induced cardiac dysfunction in mice model

The C57BL/6 mice were given intraperitoneal injections of doxorubicin with total cumulative dose of 25 mg/kg or an equal volume of normal saline. (A) The heart mass to tibia length ratio (HW/TL) between three groups. Scale bars: 1mm. (B) H&E stain and Masson trichrome stain were presented, showing pathological changes in heart tissues. Scale bars: 200μm. (C) Structural changes in the mitochondrial were assessed through TEM imaging. Scale bars: 1μm. (D) Before sacrifice, heart MRI was taken and left ventricular ejection fraction (LVEF) were measured in each group. Vales are mean ±SEM, *P<0.05, **P<0.01

13. Atorvastatin treatment prevented cardiomyocyte apoptosis in mice model

The results of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining clearly demonstrated that doxorubicin injection significantly increased apoptotic cells in mice heart, compared with mice in normal control. Moreover, the apoptotic effect of doxorubicin was decreased in atorvastatin + doxorubicin group, (Figure 13A) which indicates administration of atorvastatin contributes in reducing of apoptosis caused by cardiotoxicity induced by doxorubicin.

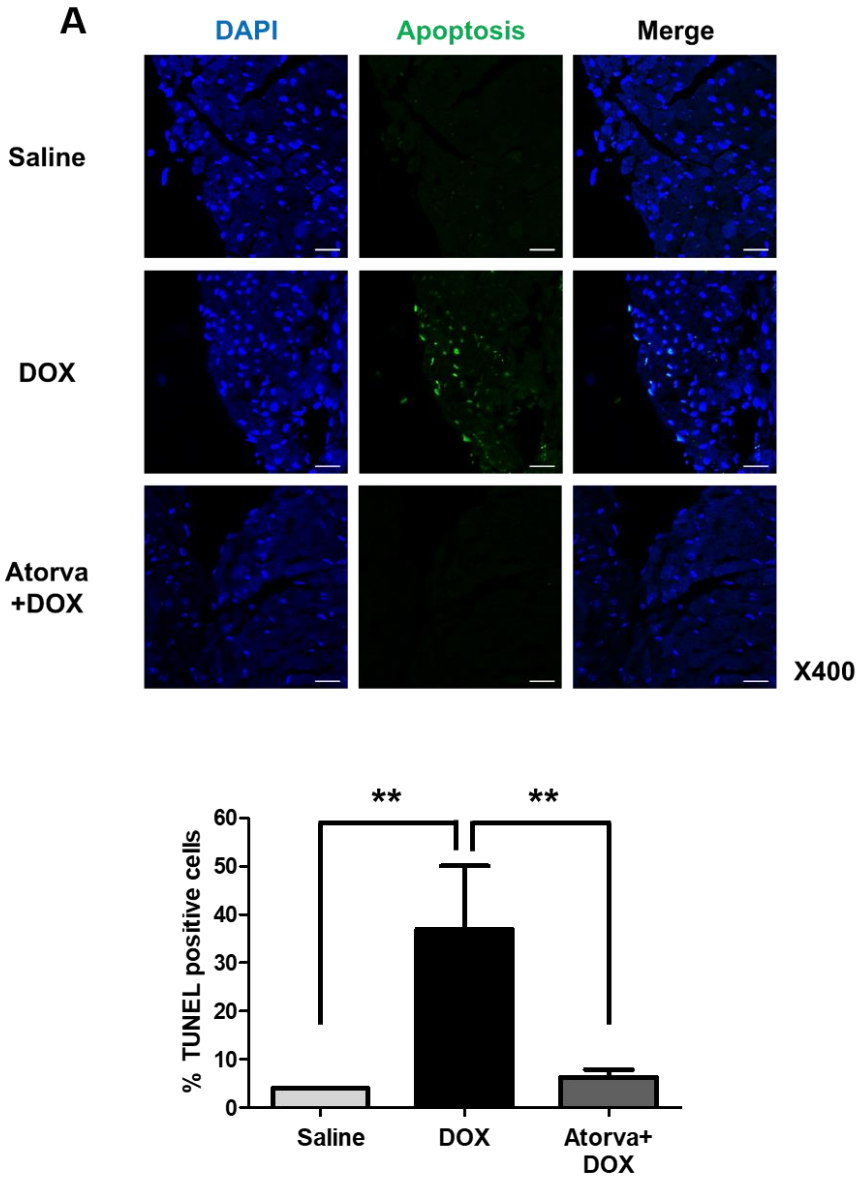


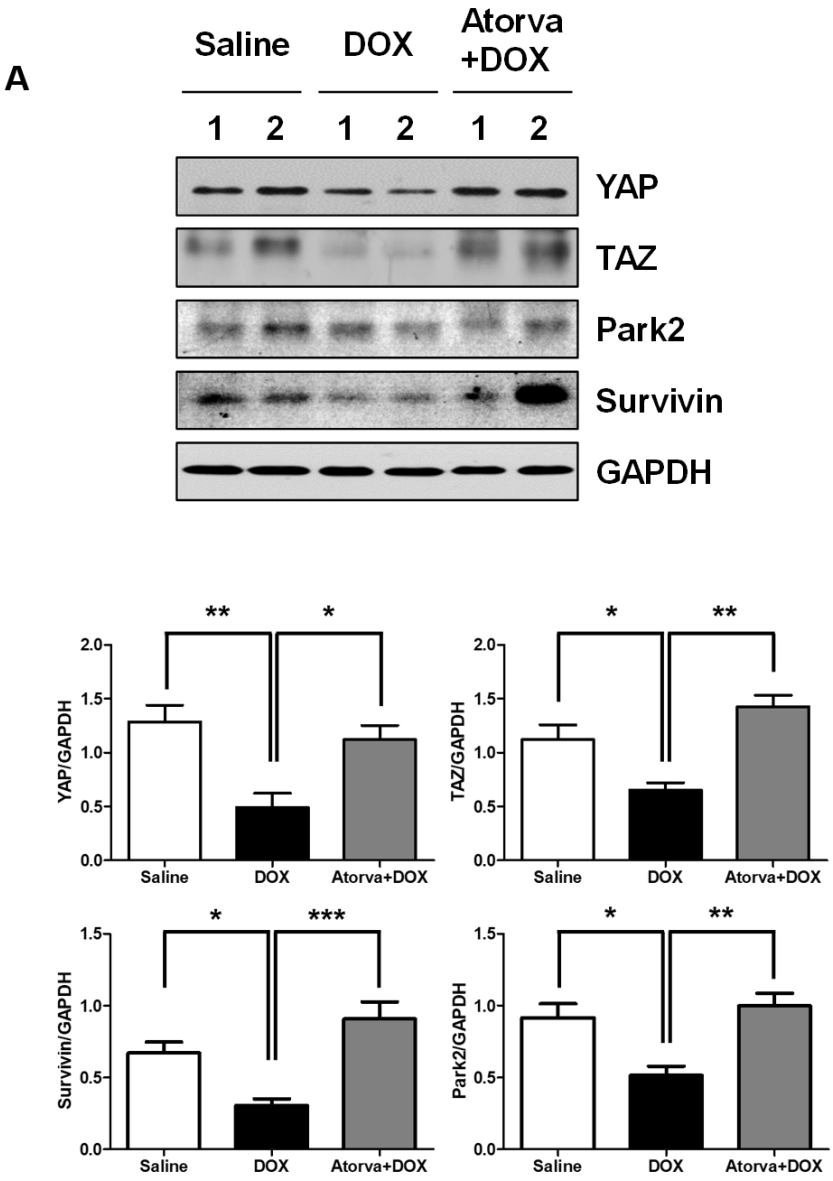
Figure 13. A legend (following pages)

Figure 13. Atorvastatin treatment improved DOX-induced cardiac toxicity via reducing apoptosis.

(A) TUNEL staining were used to detect DNA fragmentation of apoptotic cells. The middle panel represents images with TUNEL staining; the left panel displays the images with DAPI staining to demonstrate the nuclei of cells; the right panel indicates images of both to illustrate the apoptotic cells. Data are expressed as mean ± SEM, **P<0.01

14. Atorvastatin increased expression of protein associated with survival in doxorubicin-induced cardiotoxicity mice (chronic model)

To investigate the effect of statin on expression of YAP, TAZ, Park2 and survivin in doxorubicin-induced myocardial mouse model, 8-weeks-old C57BL/6 mice were daily injected with 10mg/kg of atorvastatin or saline for 6 weeks. Change of YAP, TAZ, Park2 and survivin protein was observed in western blot using the whole lysate of mice hearts (Figure 14A). And, the immunohistochemistry microscopy image also exhibited that YAP, TAZ, Park2 and survivin expression was down-regulated by doxorubicin treatment and recovered by atorvastatin co-treatment (Figure 14B- E).



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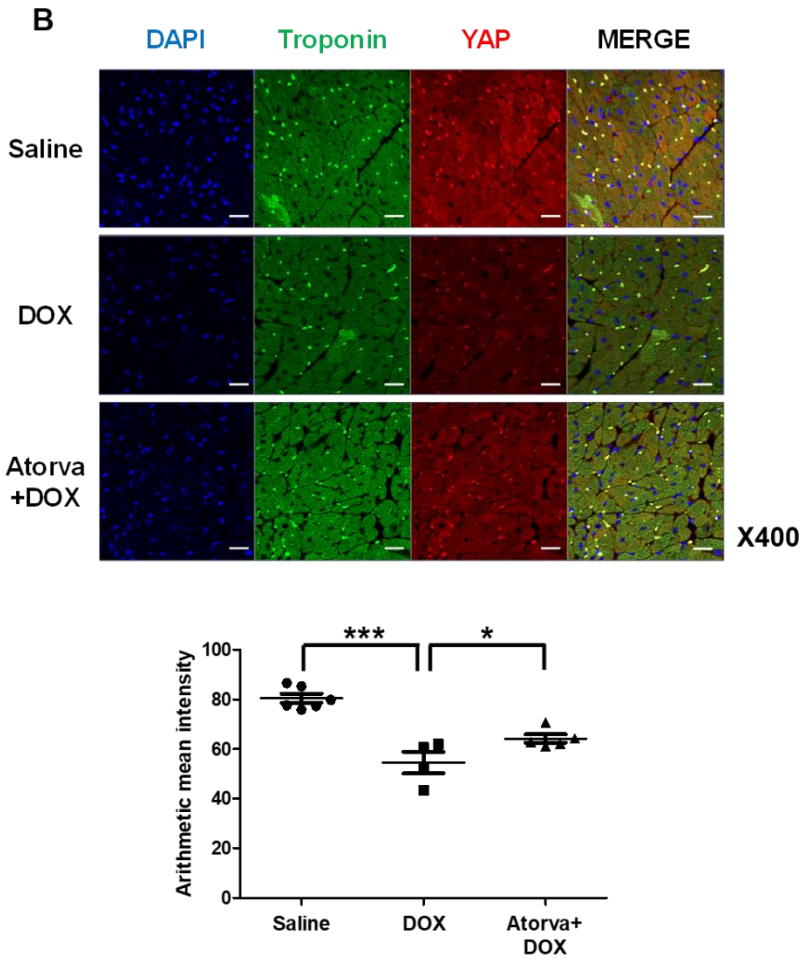

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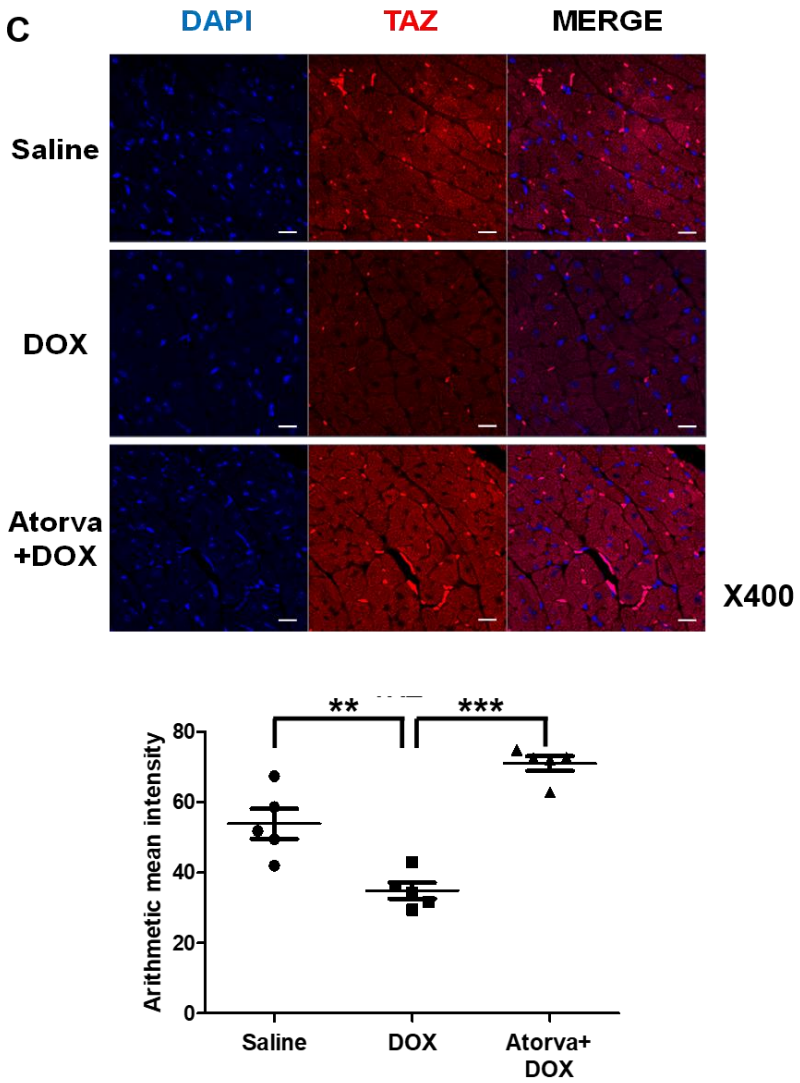

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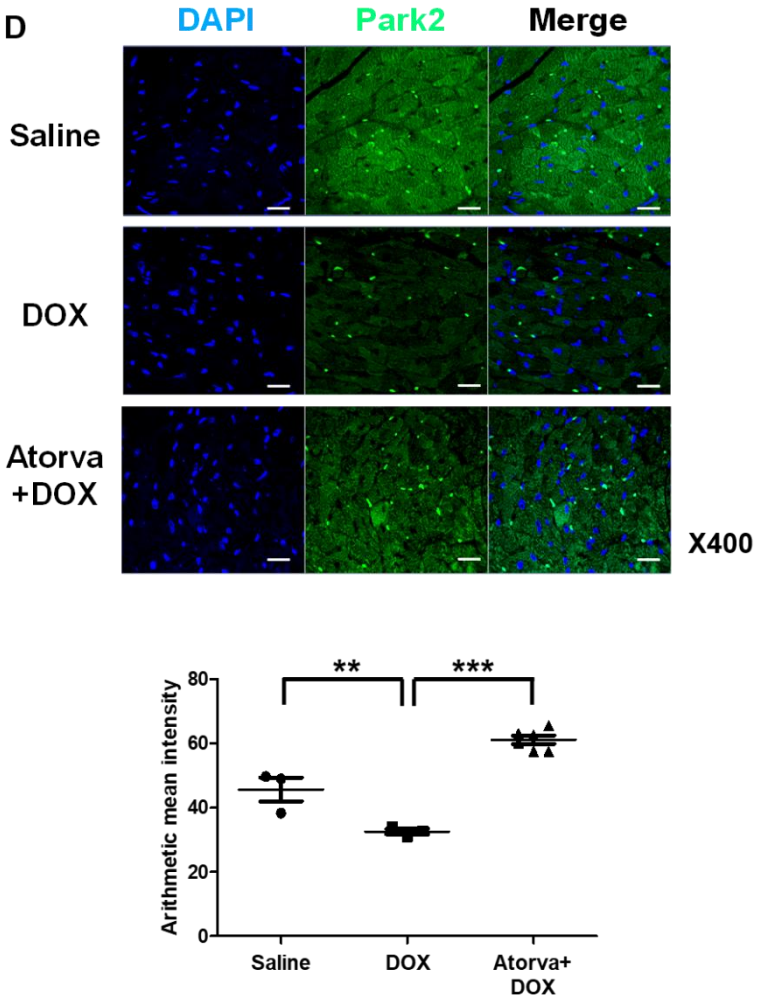

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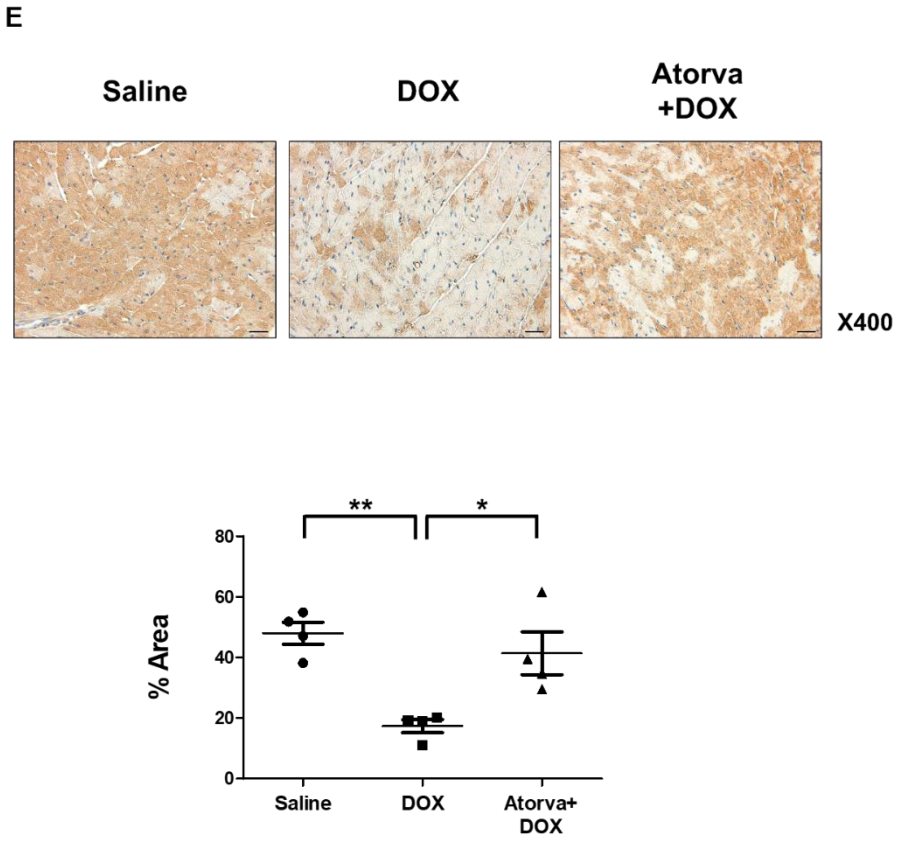


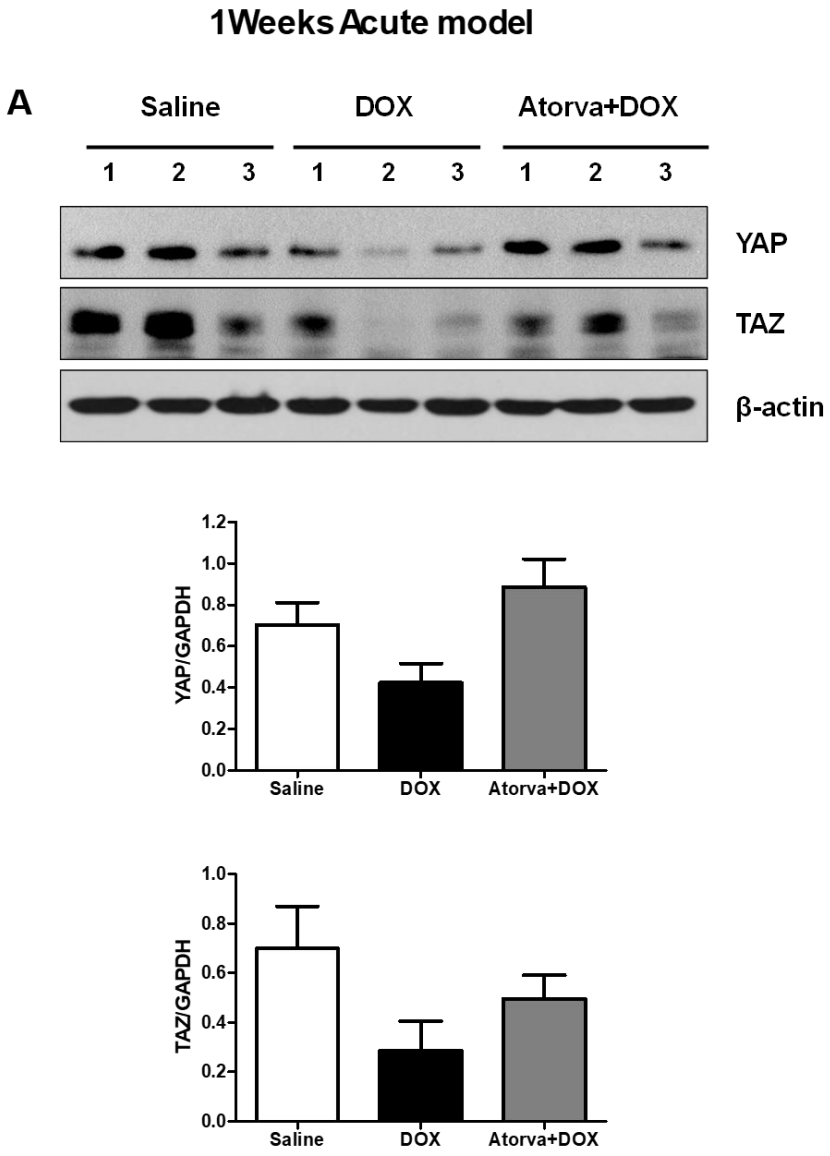
Figure 14. E legend (following pages)

Figure 14. Atorvastatin increased expression of protein associated with survival in doxorubicin-induced cardiotoxicity mice (chronic model)

(A-E) The C57BL/6 mice were treated with 5 mg/kg of doxorubicin once a week by intraperitoneal injection for 6 weeks and were treated with 10 mg/kg of atorvastatin or saline once a day by oral gavage for 6 weeks. After sacrifice, mice hearts were isolated and myocardial sections were evaluated. (A) YAP, TAZ, Park2 and survivin protein expressions were observed using western blotting analysis. (B-D) Also YAP/TAZ and Park2 was visualized by immunofluorescence, (E) survivin was visualized through DAB (3,3´ -Diaminobenzidine) staining with immunohistochemistry. Confocal immunofluorescence microscopic imaging analysis with DAPI (blue), Troponin I (green), YAP or TAZ (red), Park2 (green) in the section from mice heart (x400). Data are expressed as mean ± SEM, *P< 0.05, **P<0.01, ***p<0.001

15. Atorvastatin improved expression of protein associated with survival in doxorubicin-induced cardiotoxicity mice (acute model)

To further assess the changes in YAP, TAZ, Park2 and survivin during Doxorubicin-induced cardiomyopathy in vivo, C57BL/6 mice were intraperitoneally injected with doxorubicin, 4 times with equal doses (each containing 5 mg/kg), every 2 days with a total cumulative dose of 20 mg/kg, and the control group received the same volume of normal saline in parallel. These mice were treated with 10 mg/kg of atorvastatin or saline once a day for a week by oral gavage. Changes of YAP and TAZ expression were observed with immunoblot analysis using the whole lysate of mice hearts (Figure 15A). And, the immunohistochemistry microscopy image also exhibited that YAP/TAZ, Park2 expression were down-regulated by doxorubicin treatment and recovered by atorvastatin co-treatment (Figure 15B - D).



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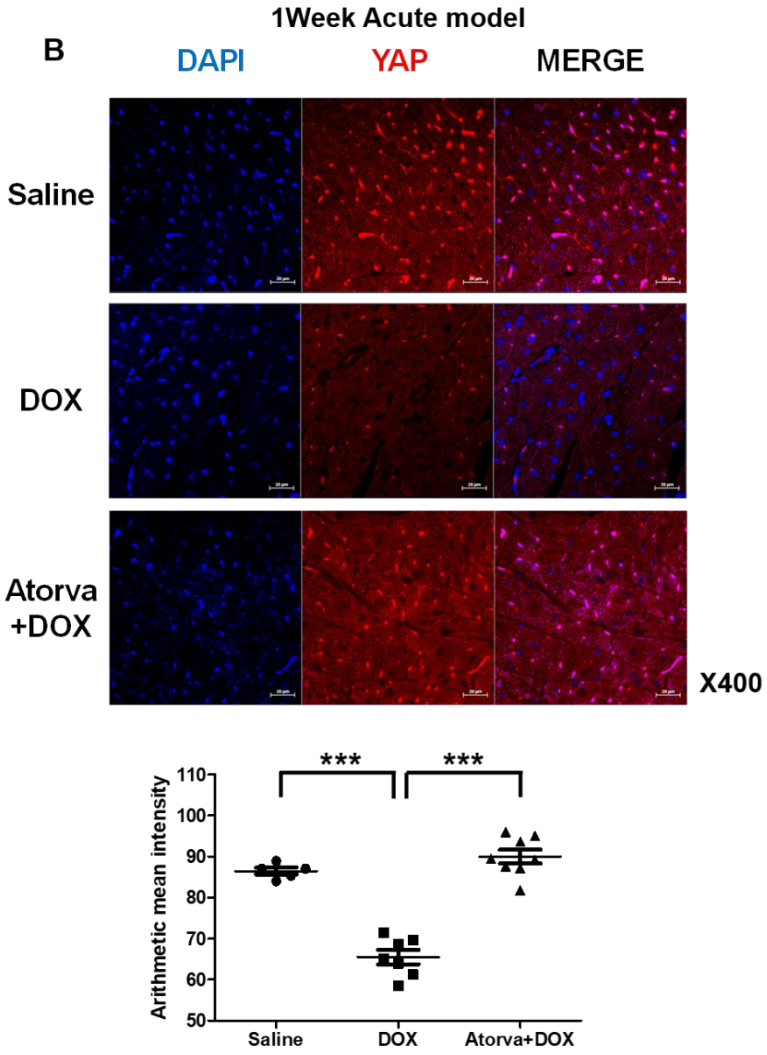

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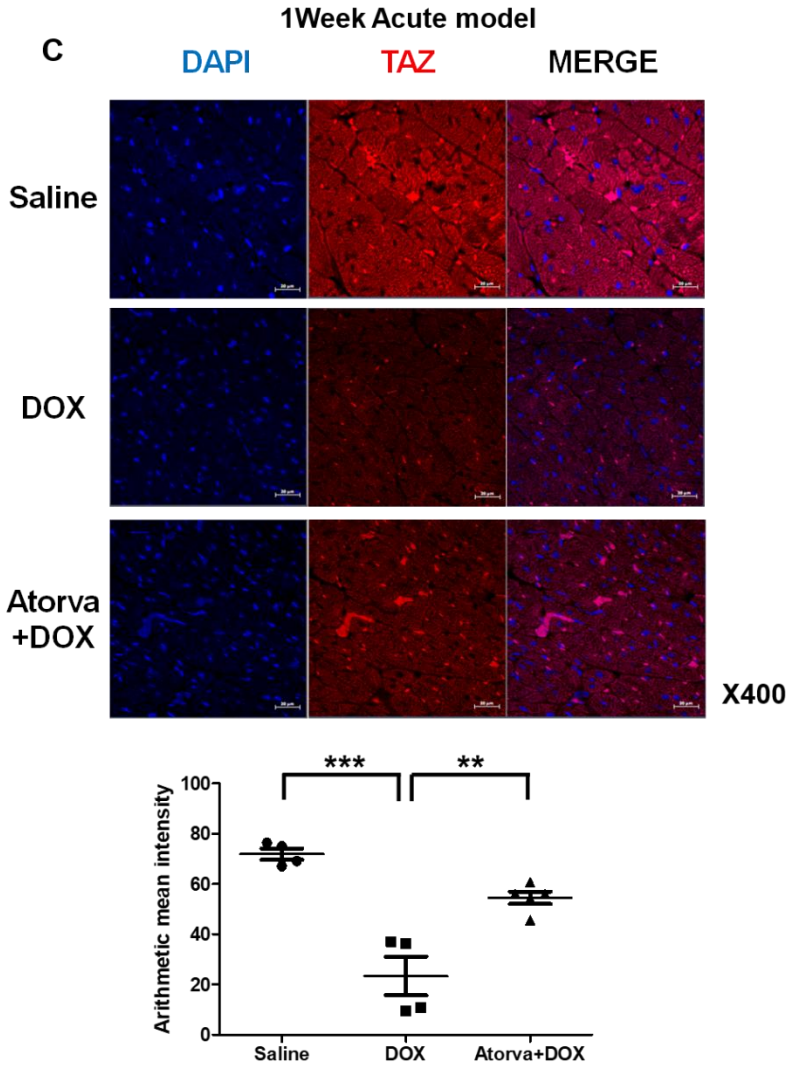

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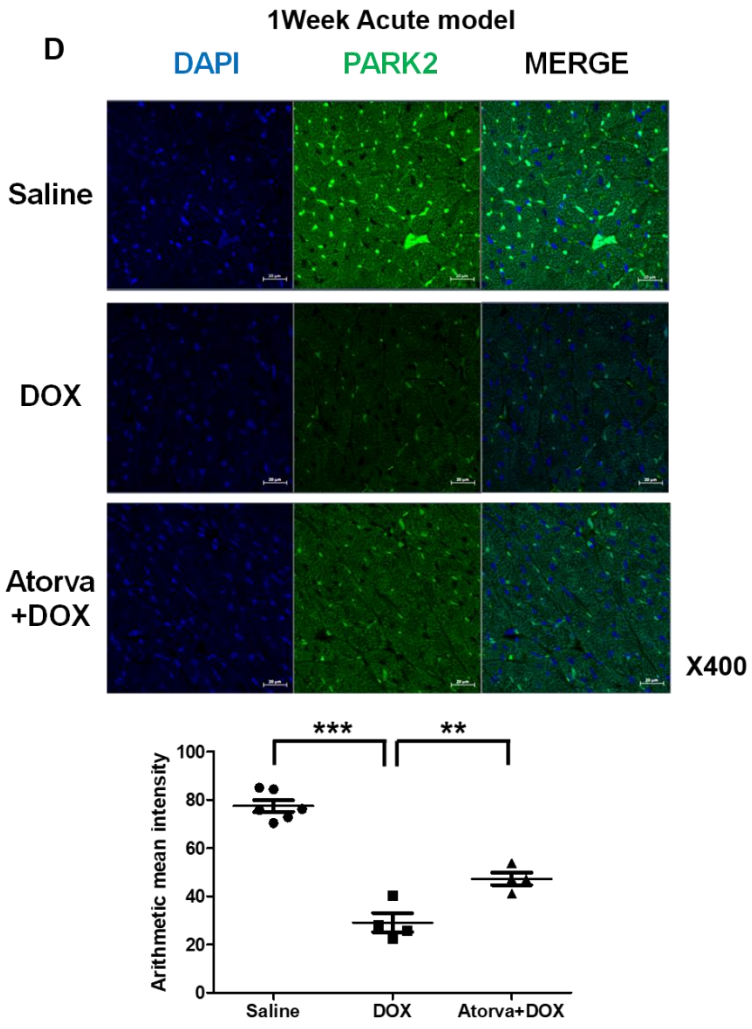

Figure 15. D legend (following pages)

Figure 15. Atorvastatin increased expression of protein associated with survival in doxorubicin-induced cardiotoxicity mice (acute model)

(A- D) The YAP and TAZ protein expression were observed using western blotting analysis and YAP/TAZ and Park2 were visualized by immunofluorescence. Confocal immunofluorescence microscopic imaging analysis with DAPI (blue), YAP or TAZ (red), Park2 (green) in the section from mice heart (x400)**.** Data are expressed as mean ± SEM, *P< 0.05, **P<0.01, ***p<0.001

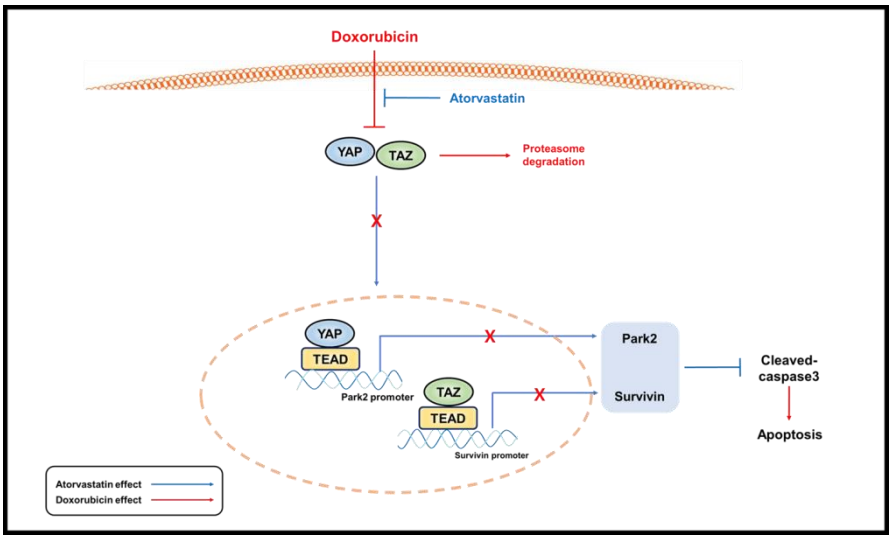

Figure 16. Atorvastatin increased expression of protein associated with survival in doxorubicin-induced cardiotoxicity mice (acute model).

Doxorubicin treatment inhibits the expression of YAP and TAZ. Atorvastatin rescued the expression of YAP and TAZ inhibited by doxorubicin. In addition, Atorvastatin reduced doxorubicin-induced cardiac toxicity by restoring Park2 and survivin, which are downstream targets of YAP and TAZ. Knockdown of YAP and TAZ regulates expression of Park2 and survivin, anti-apoptotic factor. This study suggested that, when YAP and TAZ expression are stabilized by atorvastatin, the expression of Park2 and survivin is increased and doxorubicin-induced apoptosis is alleviated.

IV. DISCUSSION

The major findings of this study are as follows. First, atorvastatin has a potential cardioprotective effect by modulating YAP and TAZ signal pathway in doxorubicin-induced cardiotoxicity in H9c2 cell and mice model. Second, Park2 and survivin mediates the cardioprotective effect of atorvastatin via the YAP and TAZ pathway.

The YAP and TAZ play an important role in regulating downstream target proteins related to apoptosis and proliferation in cancer cells treated with doxorubicin. A previous study suggested that doxorubicin-induced phosphorylation of MST1/2, LATS1, YAP, and activation of cleaved-caspase3 increased the cardiac tissue cell apoptosis[29]. Also, YAP and TAZ expression are positively correlated with Bcl2 (mitochondrial antiapoptotic pathways) expression, which shows that YAP and TAZ mediate apoptosis by regulating antiapoptotic factors.

Studies on molecular biological mechanisms about YAP and TAZ cell proliferation in anticancer drug-treated cancer cells are being actively reported[11,30,31]. Doxorubicin was exposed to cardiomyocytes, and apoptosis was induced. Similar to previous studies, the expression of YAP and TAZ were significantly reduced in doxorubicin-induced cardiomyocytes. It was also

confirmed that the YAP and TAZ protein, an activator of hippo signals, play an important role in doxorubicin-induced cardiotoxicity myocyte survival.

According to a recent study, YAP/TAZ is activated in response to doxorubicin treatment in hiPSC-CM, which increases cell proliferation, showing that cancer cells and iPSC-CMs exhibit different apoptosis profiles in response to doxorubicin. Compared with in vitro data, it was found that doxorubicin resulted in elevated caspase 3/7 levels, nuclear fragmentation, and decreased mitochondrial membrane potential in a concentration-dependent manner in hiPSC-CMs. However, overexpression of YAP suppressed apoptosis and induced proliferation, restoring doxorubicin-induced myocardial toxicity. This suggests that the role of YAP is important for hiPSC-CM cell proliferation in doxorubicin-induced cardiotoxicity[32]. We can figure that the role of YAP on apoptosis inhibition and cell proliferation in various cells is achieved via several pathways. However, YAP and TAZ mRNA levels were lower in vitro differentiated hiPSC-CM than in human ex vivo samples. In contrast, YAP expression level is high in H9c2 cardiomyocytes, but it is reduced by doxorubicin, which increases apoptotic factors and stimulates apoptosis.

However, mechanisms for preventing cardiac toxicity, a side effect of doxorubicin, are still insufficient. Therefore, studies on YAP and TAZ transcription networks that attenuate the myocardial cell apoptosis derived from

doxorubicin treatment are needed.

A recent study revealed that statins inhibit apoptosis by regulating the expression of proteins related to cell survival in cardiomyocytes treated with doxorubicin. This study suggests that survivin expression plays a pivotal role in statin to reduce cell apoptosis and increase cell survival in the doxorubicin-induced cardiotoxicity model by statin[10].

Since statins (inhibition of HMG-CoA reductase) are recognized to protect the heart from various cardiovascular diseases, including myocardial infarction and stroke, it is most widely and numerously prescribed. Recent studies have shown that inhibition of HMG-CoA reductase, a key mevalonate pathway enzyme, can regulate YAP and TAZ transcriptional activity. However, studies on the mechanism of statin protecting cardiomyocytes through YAP and TAZ in the doxorubicin-induced cardiotoxicity model have not been conducted.

So, experiments were performed to see whether statin rescues YAP and TAZ expression reduced by doxorubicin. This data shows that reduction of YAP and TAZ expression was substantially recovered through statin in doxorubicin-treated cardiotoxicity models. This was observed in parallel with elevated survivin levels as a marker to inhibit cardiomyocyte loss and caspase-3, a marker of cardiomyocyte apoptosis.

Survivin is a downstream target of YAP and TAZ signaling in cancer cells

and an essential protein in cardiac cell survival. These studies have suggested that survivin is regulated as a sub-signal protein of YAP/TAZ, which is involved in cell survival[28]. Also, in our previous study, survivin gene therapy could attenuate the progression of cardiomyocyte apoptosis in doxorubicin-induced cardiomyopathy.

It was newly explained in this study that the effect of statin on restored survivin expression was inhibited when YAP or TAZ was knockdown through transfection with siRNA. In addition, transient silencing of YAP and TAZ co-activators resulted in a related activation of cardiomyocyte apoptotic pathway (cleaved-caspase3).

It is well known that YAP regulates survivin as a transcription factor in diverse cells, including cancer cells, but the role of TAZ on modulating survivin remains unclear despite the close relationship with YAP. From this study, it was determined that TAZ knockdown significantly attenuates the expression of survivin. These findings show that not only YAP but also TAZ is essential in modulating transcriptional activation of survivin and survival of cardiomyocytes. However, current knowledge on molecular mechanisms and target proteins of YAP and TAZ signaling involved in the cardioprotective effects of statins in doxorubicin-induced cardiotoxicity is limited.

This study investigated the cardioprotective effect of statin through the

expression of Park2, as a new target of YAP and TAZ signal pathway, in doxorubicin-induced cardiotoxicity. Although the study results that YAP and TAZ regulate Park2 protein are incomplete, considering that doxorubicin causes mitochondrial damage and induces apoptosis, it was speculated that there might be a correlation.

A recent study suggested that cell apoptosis was restored and reactive oxygen species (ROS) was reduced by melatonin in doxorubicin-treated cardiomyocytes. YAP was decreased by doxorubicin and restored with melatonin treatment. In the cardiomyocyte recovery effect of melatonin, the YAP gene protects the myocardial damage by regulating survivin and Park2, which are survival proteins[33].

Another study also suggested that Simvastatin showed the cardioprotective effects by increasing Park2, and increment in infarct size was observed when ischemia/reperfusion was induced in Park2 knockout mice. However, research on regulating Park2 expression by YAP and TAZ signal pathway for the cardiac protective effect of statins has not been reported.

This study revealed that expression of Park2, reduced by doxorubicin, was preserved by statin, the knockdown of YAP attenuated these effects. However, these data were not shown in the absence of TAZ. In addition, cell apoptosis, which was reduced through statin, was increased with the knockdown of Park2.

These results suggest that cardioprotective effects of statin are established through modulation of Park2 by regulation of YAP expression. These findings are consistent with a study, which reported that Park2 is essential in recovering cardiac function and expression is regulated by YAP[34].

Data shown in vivo, expression of YAP, TAZ, Park2, and survivin were reduced in the doxorubicin-induced cardiotoxicity mice model. In contrast, the expression was enhanced in myocardium cells treated with a statin. Using cardiac MRI, we proved the cardioprotective role of statin in the doxorubicin-induced myocardial injury mouse model. This study shows that statin has a cardioprotective effect on molecular biologic, histologic, and functional measurements in vivo.

Statins are effective drugs to improve left ventricular ejection fraction (LVEF) in anthracycline-treated patients. A recent clinical study showed that statin treatment (moderate- to high-intensity doses) is associated with lower declines in (LVEF) in a woman receiving statin (trastuzumab) therapy for breast cancer. In addition, patients who took statins during cancer therapy had higher LVEF at the end of treatment and showed a smaller change in LVEF during treatment[35]. Another study showed that using rosuvastatin in breast cancer patients could prevent cardiac toxicity and preserve cardiac function[36].

Consistent with this clinical background, this study shows that YAP and

TAZ signal is a novel pathophysiological mechanism of statins that reduces doxorubicin-induced cardiac toxicity through transcriptional regulation of the antiapoptotic protein, Park2, and survivin.

However, studies on the cardiac functional role of YAP and TAZ in doxorubicin-induced cardiotoxicity animal models are insufficient. Therefore, additional studies are needed to explain the role of YAP and TAZ in cardiomyocytes using cardiac-specific knocked out or transgenic mice of YAP and TAZ genes.

In conclusion, statin is substantial in apoptosis and cell survival of doxorubicin-induced cardiotoxicity models, and in this mechanism, YAP and TAZ protein are essential factors in protecting the myocardium. Furthermore, YAP and TAZ can regulate anti-apoptosis through survivin and Park2 as cell survival proteins.

Therefore, this study will provide recent evidence for the YAP and TAZ signaling pathway mechanism by which statins inhibit myocardial apoptosis. The study of cardioprotective mechanisms through cardioprotective agents may provide better understanding of the molecular mechanisms of doxorubicin-induced myocardial toxicity.

V. CONCLUSION

These findings suggest that YAP/TAZ mediates the effects of atorvastatin on cardiac dysfunction and anti-apoptosis against doxorubicin-induced cardiotoxicity models in vitro and in vivo. Moreover, the absence of YAP and TAZ suppressed Park2 and survivin expressions restored by atorvastatin in the doxorubicin-induced cardiotoxicity model. These results suggest that YAP/TAZ regulates the expression of Park2 and survivin. In this study, new evidence is exhibited that doxorubicin-induced cardiac cell death and dysfunction can modulate cardiac protection of atorvastatin through YAP/TAZ transcription factor. Also, a new mechanism was discovered that could enhance anti-apoptosis of survivin, a key molecule of cell survival, and Park2, a new target.

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Abstract (in Korean)

독소루비신 유도 심근 독성 모델에서 YAP/TAZ 신호 전달 체계 조절을 통한 아토르바스타틴의 심근 보호 효과

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임 희 정

YAP 과 TAZ는 세포 증식 및 세포 생존에 관여하며 Hippo 신호 전달 경로에서 LATS/MST 에 의해 조절되는 전사인자이다. 또한 항암제 독성 연구에서 세포 사멸을 조절하는 중요한 역할을 한다. 독소루비신은 안트라사이클린계 항암제로서 장기간 사용시 심근 독성을 일으켜 심부전을 유발한다.

스타틴은 혈중 콜레스테롤을 낮춰서 심혈관 질환의 발생을 줄일 뿐 아니라 일부 암 환자들에서 항암제 독성을 줄이는 효과가 밝혀져 있다. YAP 과 TAZ는 전사 인자로서 세포사멸을 억제하는 능력을 가지고 있으며, 독소루비신 유도 심근 독성 유발 기전에 관여한다. 따라서 본 연구에서는 YAP 과 TAZ가 독소루비신에 의한 심장 독성에 대한 아토르바스타틴의 보호 효과에 관여하는지에 대해 조사했다.

H9c2 심근세포에 독소루비신을 처리하면 YAP 이 감소하고 그 하위 시그널인 Park2가 감소하였다. 또한 독소루비신 처리 후 TAZ가 감소하고 그 하위 시그널인 survivin이 감소하였다

반면에, 독소루비신을 처리한 H9c2 심근세포에서 감소되어 있던 YAP, TAZ, Park2 그리고 survivin은 아토르바스타틴에 의해서 mRNA와 단백질 발현이 회복되었다. 그리고 독소루비신에 의해 증가하였던 세포사멸과 이에 관련된 인자들이 아토르바스타틴에 의해서 감소하였다. 그러나 siRNA를 이용하여 YAP/TAZ의 발현을 저하시키면, 아토르바스타틴은 독소루비신으로 유도된 세포사멸을 줄이지 못하였다. 또한 독소루비신을 처리한 H9c2 심근 세포에 아토르바스타틴을 전 처리하여도, siRNA를 이용하여 YAP/TAZ의 발현을 저해 시킨

경우 Park2/survivin의 발현이 회복되지 못하였다. 게다가 siRNA를 이용하여 Park2나 survivin의 발현을 저해 시키면 아토르바스타틴은 독소루비신에 의한 세포사멸을 저해하지 못하였다.

만성 또는 급성 독소루비신 유발 심근병증 동물 모델에서 아토르바스타틴의 경구 투여는 심근 세포 사멸을 감소 시켰으며, 독소루비신으로 인해 저하된 심장기능이 회복되는 것을 cardiac MRI를 통해 확인하였다.

결론적으로 아토르바스타틴은 독소루비신으로 유도된 심장독성을 예방할 수 있었다. 이러한 아토르바스타틴의 심장 보호 작용의 기전으로는 아토르바스타틴이 YAP 및 TAZ의 발현을 증가시키고 그 하위 신호인 Park2 및 survivin을 활성화시킴으로써 항-세포사멸 효과를 나타냄을 제시한다.

핵심되는 말 : YAP, TAZ, Park2, Survivin, 스타틴, 심장독성, 독소루비신, 심근병증, 심부전