

# SENP3 protects H9C2 cells from apoptosis triggered by H/R via STAT3 pathway

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**Abstract. – OBJECTIVE:** To investigate whether SENP3 protects H9C2 cells from apoptosis triggered by H/R through the signal transducer and activator of transcription 3 (STAT3) pathway.

**MATERIALS AND METHODS:** Male C57BL mice were cultured and mouse models of myocardial I/R were established. At the same time, cardiomyoblast H9C2 cell line of rat embryo was cultured. Reactive oxygen species (ROS) level was detected during H/R using 2',7'-dichlorofluorescein diacetate (DCFH) kit. Apoptotic cells were checked by flow cytometry. The expressions of p-JAK2, JAK2, STAT3, p-STAT3, cleaved-caspase3 (c-caspase3), and Bcl/Bax were detected using Western blotting and reverse transcriptase-polymerase chain reaction (RT-PCR).

**RESULTS:** We revealed that SENP3 rose in mice of I/R group and in H9C2 cells following H/R with an increase in p-STAT3. Furthermore, increased expression of SENP3 was found to be dependent on the generation of ROS, as the SENP3 accumulation was inhibited by antioxidant (NAC). Inhibition of SENP3 suppressed the p-STAT3 expression, but promoted cell apoptosis, c-caspase3 expression, and Bcl/Bax ratio. Besides, SENP3 overexpression alleviated the cell apoptosis, which was abrogated by AG490.

**CONCLUSIONS:** SENP3 could protect H9C2 against H/R through enhancing JAK2/STAT3 pathway.

*Key Words:*

SENP3, STAT3, Apoptosis, ROS.

## Introduction

Cardiovascular disease is the most common factor causing the death of people worldwide, in which myocardial infarction ranks 1st<sup>1</sup>. Researchers have exerted great efforts to ameliorate the reperfusion therapy for acute myocardial infarction (AMI), e.g., percutaneous coronary interven-

tion (PCI), but the mortality rate remains high. Patients carrying AMI are subjected to a higher risk of developing heart failure (HF) resulting from myocardial ischemia reperfusion (MIR) lesion, one type of lesion of myocardium, as the blood flow is being recovered from the ischemic myocardium. MIR injury can be found in different areas, inclusive of coronary bypass surgery, antiplatelet antithrombotic agents, transplantation of heart, or cardiopulmonary resuscitation<sup>2</sup>, and can induce further cardiomyocyte damage on the basis of cardiomyocyte apoptosis<sup>3</sup>. Cardiomyocyte apoptosis after MIR will inhibit the cardiac function recovery in the course of revascularization treatment. Clinical experiments present a negative prospect and up to date, therapies available remain unclear in terms of the pathological mechanisms; thus, a novel target for clinical medication treatment and pharmacological design is urgently needed. The cardio-protective technique primarily aims to mobilize endogenous mechanisms capable of eliminating the damage effect of MIR<sup>4</sup>, which illustrates the cardiomyocyte apoptosis during I/R at the molecular level.

A clinical research has revealed that after MIR injury, the problem of ventricular arrhythmias first appeared and then quickly developed into an irreversible cell death triggered by apoptosis<sup>5</sup>. The main contributory factors are comprised of hypercontracture, opening of mitochondrial permeability transition pore (MPTP), calcium overload, and oxidative stress (OS)<sup>6</sup>. A recent work has reported that "survivor activating factor enhancement (SAFE)" regulated by TNF- $\alpha$  or IL-6, is bound by cardioprotection<sup>7</sup>. The signal transducer and activator of transcription 3 (STAT3) play a crucial role in cardio protection to resist at the injury of MIR<sup>8,9</sup>. Once the pathway is activated, especially the increased phosphorylation of STAT3, cell necrosis and apoptosis triggered during IR injury will be inhibited. Accordingly, scholars<sup>10</sup> have

also proved that drugs inhibit STAT3 activation, or inhibition of its transcriptional activity at the gene level, can exacerbate apoptosis and enlarge infarct size in patients suffering from MIR injury. After being activated, STAT3 can be translocated into the nucleus and its downstream target genes can be regulated through incorporation with its elements<sup>11</sup>. In this regard, STAT3 activation in cells is primarily functionalized to promote proliferation and apoptosis resistance, which expedites the malignant cell transformation in tumors, maintains the malignant phenotype of tumors<sup>12,13</sup>, and exerts a vital impact on myocardial protection during MIR injury in myocardial cells. As confirmed by a recent clinical study<sup>14</sup> on Remote Ischemic Preconditioning (RIPC) in ToF children, RIPC is able to improve heart function after surgery by enhancing phosphorylation of STAT3 expression. The cytokine family of interleukin 6 (IL-6) specifically activates the STAT3. Additionally, STAT3 is located at the JAK2 downstream, and phosphorylation of tyrosine (Tyr) 705 and serine (Ser) 727 can also activate the STAT3. Tian et al<sup>15</sup> reported that the expression levels of p-STAT3 and p-JAK in the myocardial infarction myocardial group pretreated with ischemic preconditioning were up-regulated, accompanied by an increased proportion of Bcl-2/Bax, and the outcomes were reversed after adding AG490 (JAK2 inhibitor), indicating that JAK/STAT3 signal pathway is of critical significance for myocardial protection.

OS plays a crucial role in cell damage in the course of MIR, which leads to the necrosis, apoptosis, and impairment of cardiac function. In the preliminary reperfusion, reactive oxygen species (ROS) is accumulated, as energy substrates are lost in myocardial ischemia. ROS is deemed as an important intracellular messenger in gene regulation and cell signal transduction, according to numerous researches. In pathological conditions, ROS eventually leads to oxidative stress damage as ROS production is excessively accumulated, or resulting from the inhibition of its own degradation pathway. OS affects the normal structure and function by oxidizing cell membranes, lipids, proteins, and even DNA, leading to cell death ultimately. OS in the heart is correspondingly risen following heart failure, hypertrophy, and IR injury<sup>16</sup>. As confirmed in the previous researches<sup>17</sup>, a sentrin/SUMO2/3-specific protease, SENP3, is regarded as a molecule susceptible to redox and able to be accumulated in the case of cellular oxidative stress. Through eliminating the

amelioration of SUMO2/3, the functions of the intracellular substrates of SENP3 are regulated by the raised amount of SENP3. Accordingly, the epithelial-mesenchymal transition, angiogenesis, tumorigenesis, and cell proliferation will be enhanced<sup>18</sup>. SENP3 accumulation has also been found under oxidative stress and can regulate STAT3 activity by interacting with STAT3 and impeding SUMO2/3 to proliferate cells<sup>19</sup>. Our preliminary data demonstrated the accumulation of SENP3 in H9C2 cells as suffering from hypoxia/re-oxygenation (simulating MIR *in vitro*). We aim at clarifying whether SENP3 inhibits the apoptosis of H9C2 cells triggered by H/R through the STAT3 pathway.

## Materials and Methods

### *Animals*

C57BL mice (8-10 weeks old) were purchased from the Experimental Animal Center, School of Medicine, Shanghai Jiao Tong University (Shanghai, China). In this experiment, the mice were cultured with a standard diet in a stable environment in an animal room of specific pathogen-free (SPF) laboratory (12 h light/dark cycle, 40-50% humidity at 20-23°C). This study was approved by the Animal Ethics Committee of Shanghai Jiao Tong University Animal Center.

### *Myocardial I/RI in Mouse*

The mice in this study were immersed with isoflurane using a rodent ventilator and anesthetized (model 683, Harvard Apparatus, Inc. Holliston, MA, USA), and the mice were kept warm via a heating pad (preserved body temperature ranging from 36°C to 37°C). Subsequently, the mice were incised at their fourth intercostal space, and the blood flow was obstructed through ligating the left coronary artery (LAD) at the anterior descending branch, adopting 7-0 nylon suture, and introducing a silicon tubing (1 mm outside diameter) 2 mm under the border between left ventricular (LV) and the left atrium at the LAD top. ECG changes (ST elevation) confirmed the local ischemia. Finally, the silicon tube was removed at 2 h after occluding the blood for 45 min.

### *Cell Culture and Establishment of H/R Models*

American Type Culture Collection (ATCC, Manassas, VA, USA) offered the cardiomyoblast H9C2 cell line of rat embryo. Cardiomyoblast

H9C2 cell line was regularly cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island, NY, USA) containing 100 µg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA), 100 U/mL penicillin, and 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), and then incubated at 37°C through adopting 5% CO<sub>2</sub>. After that, the complete medium was replaced with the medium including 0.5% FBS for 12 h prior to harvest as cells were appropriately confluent (80%). When needed, NAC was pre-treated for 4 h at 50 µM and AG490 was pre-administrated for 1 h. We incubated H9C2 cells in serum-free Dulbecco's modified eagle medium of an airtight incubating tank for 12 h in the concentration of oxygen < 1%, with various periods of subsequent re-oxygenation (1,2,4,8 h) to trigger H/R injury. This treatment carried out outside the living organism simulates the myocardial IRI in the living organism.

#### **Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)**

In this experiment, heart tissues were cut into slices, and the overall RNA was isolated via TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the instructions. On this basis, through adopting the First Strand complementary Deoxyribose Nucleic Acid (cDNA) Synthesis Kit (TaKaRa, Dalian, China), we reversely transcribed the isolated RNA to cDNA. Through adopting the ABI Prism 7500 system with SYBR Green (Roche, Basel, Switzerland) employed, we quantified the cDNAs as instructed by manufacturer. Data were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcript levels. Life Technologies (Invitrogen, Carlsbad, CA, USA) fabricated the primers, and the sequences were originated from a NCBI database for mouse GAPDH and SENP3. The primers used in this study were shown below: SENP3: (reverse) 5'-GCGAGGTGCCTTTTTGAGTTAGAG-3'; (forward) 5'-CCGGCCATCTTTTGATGACCTT-3'; GAPDH: (reverse) 5'-AGTGTAGC-CCAAGATGCCCTTC-3'; (forward) 5'-AATGTGTCCGTCGTGGATCTGA-3'.

#### **Reactive Oxygen Species (ROS) Detection**

In this experiment, the 2',7'-dichlorofluorescein diacetate (DCFH-DA; Sigma-Aldrich, St. Louis, MO, USA) was adopted as reagent to capture ROS. H9C2 cells were inoculated into a six-well plate with the concentration of approximately 60-70%, and DCFH-DA was added with a final

concentration of 10 µM for 30 min at 37°C in an incubator. The cells were re-suspended, followed by washing with cold phosphate-buffered saline (PBS), based on which the fluorescence intensity of DCF by flow cytometry was ascertained.

#### **Transfection**

H9C2 cells were inoculated to a six-well plate at approximately concentration of 45-55% prior to the transfection. The SENP3 siRNA and negative control (NC) were ordered from GenePharma (Shanghai, China), and plasmid SENP3 was offered by Transheep Bio-Tech Co., Ltd., (Shanghai, China). The cells were transfected with plasmid SENP3 siRNA (100 nM) and SENP3 via lipofectamine 3000 (Thermo Fisher, Waltham, MA, USA) for 4 h. Subsequently, the cells were added to Dulbecco's modified Eagle's medium containing FBS for 48 h with Vector or NC as a control. Additionally, this experiment ascertained the transfection efficiency through adopting Western blot.

#### **Detection of Apoptotic Cells with Flow Cytometry**

The work ascertained the programmed death of H9C2 cells triggered by H/R in through adopting an Annexin V-FITC/PI kit (Ebioscience, San Diego, CA, USA) abiding by the flow cytometry. Briefly, the cells were washed adopting PBS chilled, and subsequently trypsin was given to the cells, which were re-suspended in 200 µL binding buffer. The cells were labeled with 10 µL propidium iodide (PI) and 5 µL Annexin V-FITC solution at the normal temperature inside a house for 10 min in the dark. To examine the fluorescent signals, flow cytometry was used (Becton-Dickinson, San Jose, CA, USA).

#### **Western Blotting Analysis**

The cells were collected to extract proteins. Whole-cell lysates were prepared through employing ice-cold RIPA buffer (Beyotime Biotechnology, Shanghai, China) containing protease inhibitor (Cocktail tablets, Roche Applied Science, Mannheim, Germany). The results ascertained the concentrations of protein through adopting the bicinchoninic acid (BCA) kit for assessing protein (Pierce Biotechnology, Waltham, MA, USA). Through adopting 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the extracted proteins were separated and loaded. Afterwards, these proteins were transferred polyvinylidene difluoride

(PVDF) membranes (Millipore, Billerica, MA, USA). Then, the membranes were cultured overnight at 4°C with the corresponding antibodies. The antibodies resisting GAPDH and SENP3 (Cell Signaling Technology, Danvers, MA, USA) and the antibodies resisting cleaved-caspase-3, Bax, Bcl2, p-JAK2, JAK2, p-STAT3, and STAT3 (Abcam, Cambridge, MA, USA) were adopted. The goat anti-rabbit IgG (Abcam, Cambridge, MA, USA) was added at room temperature for 2 h. Through adopting electrochemiluminescence (ECL) plus kit (Millipore, Billerica, MA, USA), the blots were visualized. The relative protein levels were normalized to GAPDH.

### Statistical Analysis

For no less than 3 independent experiments, all quantitative data were expressed as mean ± standard deviation. SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Comparison between groups was conducted using One-way ANOVA, followed by Post-Hoc Test (Least Significant Difference).  $p < 0.05$  represented that the difference was statistically significant.

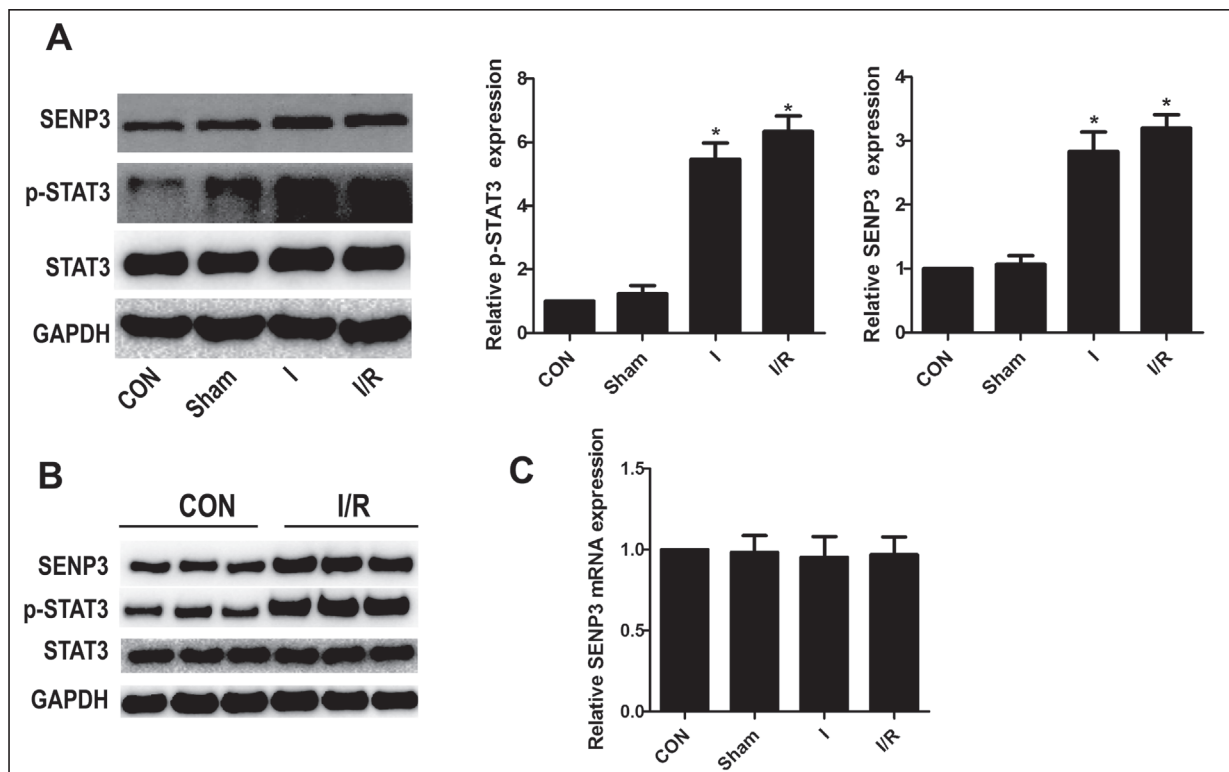
## Results

### SENP3 Expression Level was Increased in Myocardial Tissues of Mice Following I/R

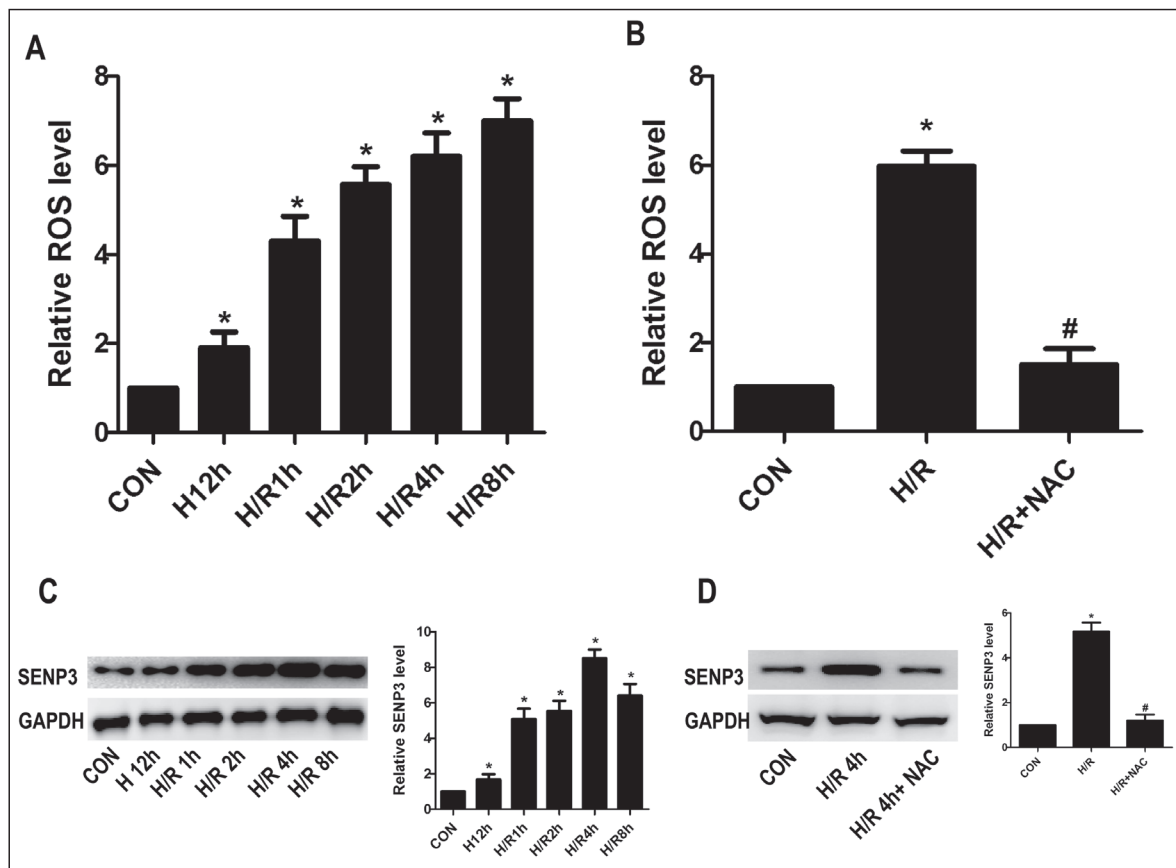
To investigate whether SENP3 participated in the protective effect from apoptosis triggered by IRI, the SENP3 expression level was examined in the myocardium of mice subject to I/R operation (Figure 1A). SENP3 expression level in reperfusion group rose evidently in contrast to that of Sham or CON group, which was accompanied by an increased expression of p-STAT3. The results were even observed in the ischemic group. Yet we did not find there was an evident change in the mRNA level of SENP3 (Figure 1C).

### SENP3 Expression Level Rose in H9C2 Cell Follow H/R Mediated by ROS

After treatment of re-oxygenation (1, 2, 4 or 8 h) followed by 12 h of hypoxia, the ROS in H9C2 cells generated during H/R was detected. DCFH-DA can be oxidized to DCF by ROS in the cell, so the fluorescence intensity of DCF represents the ROS level. The ascertainment of the relative ROS level was indicated as an increase



**Figure 1.** The expression of SENP3 in myocardial tissue of mice following I/R. **A, B**, Protein expression of SENP3, STAT3, and p-STAT3 in myocardial tissue following I/R injury was measured by Western blot. **C**, The mRNA level of SENP3 was detected by RT-PCR. \* $p < 0.05$  vs. CON group.



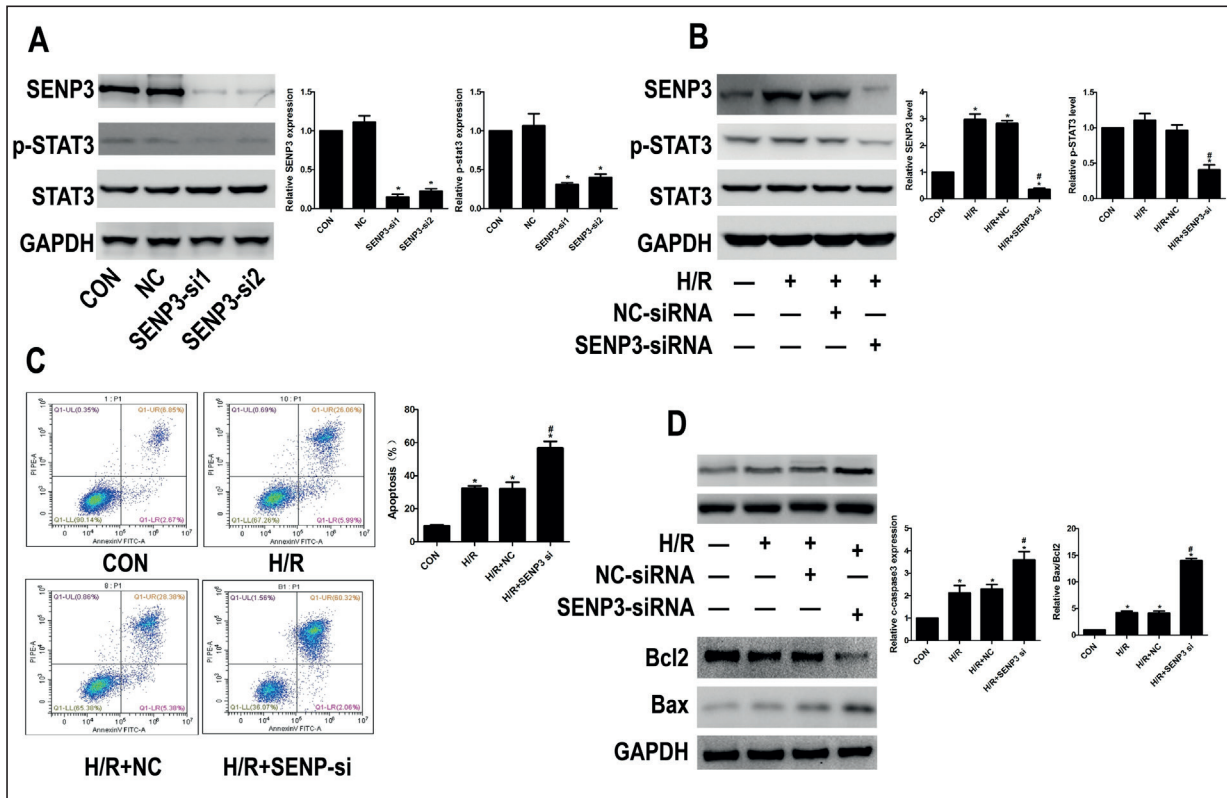
**Figure 2.** The expression level of SENP3 was increased in H9C2 cell follow H/R mediated by ROS. **A, B,** ROS level was determined by DCHF-DA staining. **C,** The protein expression of SENP3 after 12 h hypoxia and various periods of reoxygenation in H9C2. **D,** The expression of SENP3 in H9C2 pretreated with NAC suffered to H 12 h/R 4 h. \* $p < 0.05$  vs. CON group. # $p < 0.05$  vs. H/R group.

depending on time in the course of H/R, and the expression of SENP3 was elevated in H and H/R group simultaneously. Particularly when H9C2 cells were subjected to 12h H/ 4h R, the SENP3 expression level reached the highest level, so the 12 h of hypoxia followed by 4 h of re-oxygenation was selected for further study on H/R injury. The increased expression of SENP3, as a result of cysteine oxidation, could be obstructed by N-acetyl-L-cysteine (NAC). H9C2 cells were pretreated with NAC prior to re-oxygenation, and then the accumulation of SENP3 was abolished by the pretreatment (Figure 2D).

#### **Knockdown of SENP3 Aggravated Cell Apoptosis Triggered by H/R**

To probe into the impact exerted by SENP3 on H/R injury in H9C2, the SENP3 expression was knocked down, and subsequently the apoptosis rate, the expression of cleaved-caspase3 (c-caspase3), Bcl2, and Bax were detected. First-

ly, SENP3 was stably knocked down through adopting siRNA with a decline in the expression of p-STAT3 simultaneously (Figure 3A) at the basic level. Although the SENP3 expression rose in Group H/R, the p-STAT3 in the group remained unchanged in contrast to CON group. Yet the p-STAT3 expression level was evidently dropped in the SENP3-si + H/R group in contrast to the NC + H/R group (Figure 3B). The c-caspase3 expression level in group H/R surmounted that of group CON evidently, and the knock down of SENP3 further increased the expression of c-Caspase3 in contrast to the NC + H/R group (Figure 3D). Furthermore, similar results were observed in detection of apoptosis by flow cytometry that low expression of SENP3 aggravated apoptosis of H9C2 subjected to H/R (Figure 3C). In addition, the depletion of SENP3 reduced the expression ratio of Bax/Bcl2 (Figure 3D), which indicated that knockdown of SENP3 promoted cell apoptosis triggered by H/R in H9C2 cells.



**Figure 3.** Downregulation of SENP3 aggravated cell apoptosis induced by H/R in JAK2/STAT3 pathway dependent way. *A*, The expression of SENP3 in H9C2 transfected with NC or SENP3-siRNA was determined by Western blot. *B*, Representative blot of SENP3 and p-STAT3 levels in H9C2 suffered H/R were assayed by Western blot. *C*, H9C2 cell apoptosis was evaluated by flow cytometry. *D*, Representative blot of c-caspase3 and Bax/Bcl2 ratio were detected by Western blot. \* $p < 0.05$  vs. CON group. # $p < 0.05$  vs. H/R + NC group.

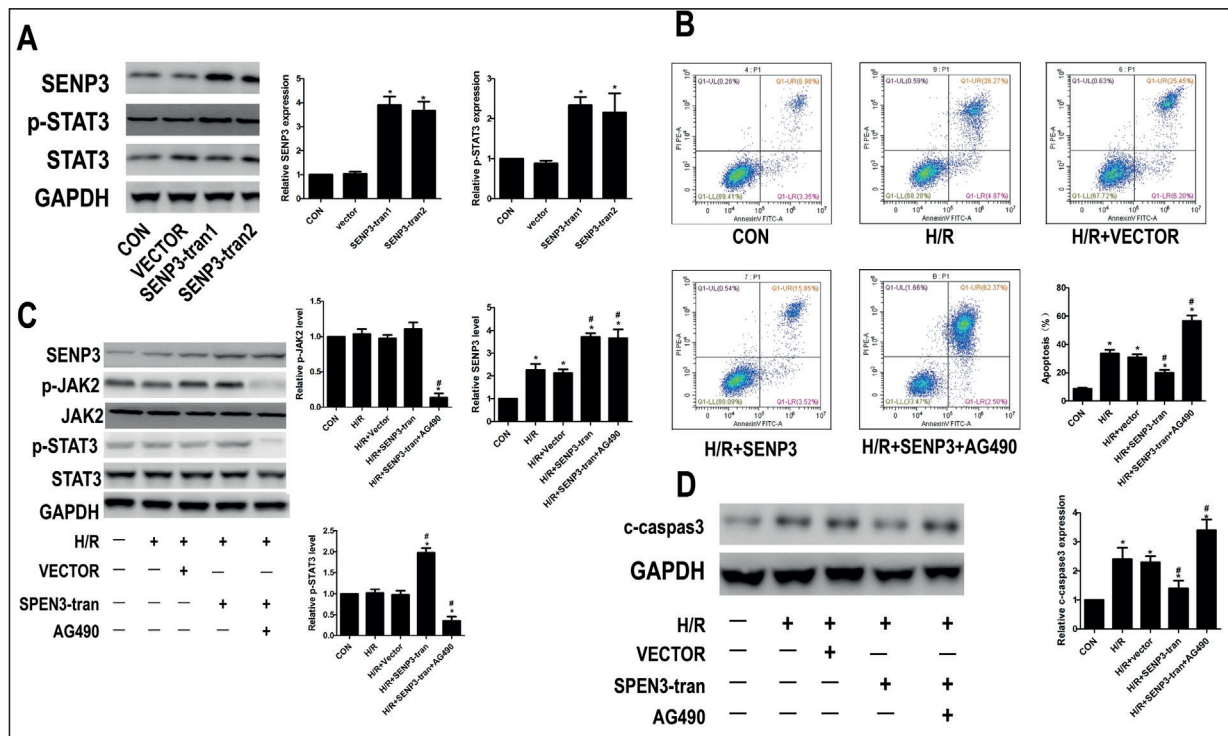
**SENP3 Protected H9C2 Cells Against Apoptosis Triggered by H/R Injury Through Enhancing STAT3 Phosphorylation**

Previous evidence demonstrated that JAK2/STAT3 pathway participates in multiple processes in heart. To determine whether the JAK2/STAT3 pathway is involved in anti-apoptotic effect mediated by SENP3 in H/R injury, H9C2 cells were stably overexpressed of SENP3 resulting in an enhancement of STAT3 phosphorylation (Figure 4A). Moreover, the protein levels of SENP3 and p-STAT3 were significantly elevated in SENP3 + H/R group in contrast to vector + H/R group (Figure 4C). Further, we found that AG490 significantly reversed the effects of SENP3-induced upregulation of p-STAT3 (Figure 4C). The cell apoptosis and the expression of c-caspase3 were assessed afterwards. Overexpression of SENP3 consequently dropped the apoptosis in SENP3 + H/R group in contrast to vector + H/R group (Figure 4D). Yet AG490 abrogated the inhibitory

effect exerted by SENP3 on apoptosis. As expected, similar results were found regarding the expression of c-caspase3. The overexpression of SENP3 resulted in a reduction of c-caspase3 expression. Moreover, AG490 abolished the inhibitory effects of SENP3 on c-caspase3 reduction. Taken above, SENP3 alleviated apoptosis triggered by H/R in a JAK2/STAT3 dependent way.

**Discussion**

Remodeling, contractile dysfunction, arrhythmias and myocardial metabolic disorders are involved in the pathogenesis of myocardial I/R injury<sup>20</sup>. MIR may induce a large accumulation of ROS via multiple mechanisms, resulting in lethal arrhythmias, myocardial stunning and microvascular damage<sup>21</sup>. The JAK2/STAT3 signal pathway serves as an axis to protect the heart, brain, kidney and liver<sup>22-25</sup>. This work surveyed whether SENP3 regulates STAT3's protection of the myocardium



**Figure 4.** SENP3 protected H9C2 cells against apoptosis induced by H/R injury through enhancing STAT3 phosphorylation. **A**, The expression of SENP3 in H9C2 transfected with vector or plasmid-encoding SENP3 was determined by Western blot. **B**, Representative blot of SENP3 and p-STAT3 levels in H9C2 suffered H/R were analyzed by Western blot. **C**, H9C2 cell apoptosis was evaluated by flow cytometry after stably overexpression of SENP3. **D**, Representative blot of c-caspase3 ratio were detected by Western blot. \* $p < 0.05$  vs. CON group. # $p < 0.05$  vs. H/R + vector group.

from apoptosis during H/R in H9C2, as well as its potential mechanism. The results demonstrated that the generation of ROS during reperfusion or hypoxia resulted in the accumulation of SENP3 in myocardial tissues or H9C2. Additionally, the silencing of SENP3 expression was accompanied by a declined expression of p-STAT, thus exacerbating the apoptosis of H9C2 triggered by H/R. Furthermore, the overexpression of SENP3 enhanced the phosphorylation of STAT3, which could protect H9C2 from apoptosis triggered by H/R.

OS is a factor known for ischemia-reperfusion injury. It is characterized by the imbalance between the antioxidant defense system and the generation of ROS<sup>16</sup>. ROS regulates the activation of signaling molecules, including mitogen-activated protein kinases (MAPK), activating protein-1 (AP-1) and nuclear factor-kappa beta (NF $\kappa$ β). “Redox signaling” defines the mediation of signaling pathways by ROS<sup>16</sup>. OS treatment is of great significance in the prevention of such injury, which is consistent with the above-mentioned observations<sup>27</sup>. However, both clinical and experimental studies<sup>3</sup> have manifested that there

are controversial results in antioxidant therapy at the onset of myocardial reperfusion. The underlying reasons may be the low efficiency of the drug permeating cytomembrane, or that the drug itself inhibits the compensatory protective mechanism triggered by ROS. A cell culture model of H/R revealed that ROS inhibited the apoptosis, suggesting that redox can regulate pathways like JNK and promote the survival of cardiac myocytes<sup>28</sup>. This study illustrated that the SENP3 expression rose in the myocardium of I/R group, and even in the ischemia group. The similar results were also observed in H9C2 treated with H/R. Accordingly, the generation of ROS during H/R or even hypoxia could increase the accumulation of SENP3, which could be blocked by anti-oxidants such as N-acetylcysteine (NAC). Since the level of mRNA did not change significantly in myocardium of each group, we hypothesized that the accumulation was achieved not by raised transcription level but probably by reductive degradation through inhibiting the ubiquitin-proteasome pathway<sup>17</sup>. This speculation requires to be further confirmed in H9C2 whereby researches.

Then SENP3 was knocked down and overexpressed in H9C2 cells using siRNA and overexpression transfection technique. The results demonstrated that when SENP3 was knocked down, the expression of p-STAT3 was decreased, and vice versa. Additionally, similar results were observed during H/R.

STAT3 plays a primary role in myocardial protection, which has attracted more concerns<sup>29</sup>. The cardio-protective effect exerted by STAT3 activation has been confirmed. However, the results of the phosphorylation extent of STAT3 in the myocardium of I/R or in H9C2 after H/R are controversy. Some studies have revealed that the phosphorylation level of STAT3 is upregulated in ischemic re-perfused myocardium, whereas those in others are reduced. In this study, no evident difference was found in the phosphorylation level of STAT3 between H/R group and control group, whereas the p-STAT3 expression dropped notably as SENP3 was knocked down in H9C2 after H/R. To further understand whether SENP3 regulated the activity of STAT3 through JAK/STAT3 pathway, AG490 (JAK inhibitor) was added after the expression of SENP3 was upregulated. In addition, the results of this study indicated that the increased expression of p-STAT3 triggered by the accumulation of SENP3 was inhibited by AG490. These findings are consistent with those of other previous studies<sup>30,31</sup>.

SENP3 expression triggered by ROS generated during H/R in H9C2 cells was detected, and the role of SENP3 in the regulation of cell apoptosis was ascertained in this study. Besides, this study manifested that the depletion of SENP3 effectively promoted cell apoptosis, and overexpression of SENP3 could suppress cell apoptosis by regulation of the activity of STAT3, which corresponded to the expression of c-caspase3. When AG490 was added, the protective effect of SENP3 against apoptosis was counteracted. The expression ratio of Bax/Bcl2 is regulated by knocking down SENP3, which also demonstrated a potential signaling mechanism of anti-apoptotic effect of SENP3.

### Conclusions

We have demonstrated that SENP3 is regarded as a crucial mediator of the cellular response during H/R. Stimulating ROS produced during H/R leads to the accumulation of SENP3 in cells, which exerts anti-apoptotic effects. The ac-

quired results ascertained that SENP3 triggered phosphorylation of STAT3, with up-regulation of the Bcl-2/Bax expression and inactivation of caspase-3. These findings insert novel mechanistic insights into SENP3 and trigger cardio protection, which may be conducive to enriching the therapeutic utility of target drugs in limiting myocardial infarction and apoptosis after I/R injury.

### Acknowledgements

This work was supported by the National Nature Science Foundation of China (No. 81670225).

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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