Lnc-PXMP4-2-4 alleviates myocardial cell damage by activating the JAK2/STAT3 signaling pathway

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A B S T R A C T

Purpose: The aim of this study was to investigate the protective effect of long non-coding lnc-PXMP4-2-4 on myocardial cell damage caused by acute myocardial infarction (AMI).
Methods: Peripheral blood mononuclear cells (PBMC) were collected from 24 patients with AMI on the day of admission, the first day after percutaneous coronary intervention (PCI) and the third day after surgery, and 24 patients with clinical control group. Real-time quantitative PCR(QRT-PCR) was used to detect the expression of related genes. Then in human cardiomyocytes (AC16), Cell Counting Kit-8 (CCK-8) was used to determine cell viability, lactate dehydrogenase release assay (LDH) was used to determine the release of lactate dehydrogenase, PCR was used to detect the expression of genes, cell death was detected by flow cytometry, and the expression of related proteins was measured by Western blot. The effect of lnc-PXMP4-2-4 was further studied by silencing and overexpressing lnc-PXMP4-2-4.
Results: Compared with clinical control group, the expression of lnc-PXMP4-2-4 in PBMC of AMI patients was significantly higher than it. Compared with pre-operation, the expression of lnc-PXMP4-2-4 was significantly up-regulated on day 1 after PCI, and recovered to pre-operation level on day 3 after surgery. In AC16 cells, lnc-PXMP4-2-4 inhibited the proliferation of AC16, promoted the release of LDH and increased cell death, aggravated the cardiomyocyte injury caused by H_2O_2, and inhibited the expression of JAK2 and STAT3 mRNA and protein. The up-regulation of lnc-PXMP-4-2-4 had the opposite effect. In addition, the inhibition of the signal pathway by JAK2/STAT3 pathway inhibitor AG490 partially weakened the enhanced viability of AC16 cells, decreased LDH release and apoptosis induced by lnc-PXMP4-2-4 overexpression, increased Bcl-2 expression and down-regulated Bax expression.
Conclusion: Therefore, we conclude that lnc-PXMP4-2-4 protects cardiomyocytes from injury by activating the JAK2/STAT3 signaling pathway.

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1. Introduction

Cardiovascular disease (CAD) is the main cause of death in the world [1], of which acute myocardial infarction (AMI) annual death toll of nearly 17.8 million, for a long time occupied the first of various death factors [2,3], bringing a great burden to medical care. Although percutaneous coronary intervention (PCI) significantly improves the survival rate of AMI [4], ischemia-reperfusion is prone to cause oxidative stress, which will further aggravate myocardial cell damage [5,6] and lead to a series of complications and adverse clinical outcomes. Therefore, there is an urgent need for a new therapeutic approach to alleviate myocardial cell damage caused by AMI.

Long non-coding RNAs (lncRNA) are a class of RNA transcripts that are more than 200 nucleotides in length [7,8]. In the cardiovascular system, studies have characterized the expression of LncRNA in normal physiological and disease states [9]. Multiple lncRNAs are regulated in the course of acute myocardial infarction and heart failure [10,11], and they are emerging as potential key therapeutic targets for multiple cardiovascular diseases [12,13]. However, the changes and roles of LncRNA in myocardial infarction remain unclear to a large extent, and it is particularly important to explore the relevant mechanisms. lnc-PXMP4-2-4 is a member of antisense RNA in the peroxisome membrane protein family. A sarch of NONCODE (http://www.noncode.org/) and LNCipedia (https://lncipedia.org/) showed that lnc-PXMP4-2-4 was differentially expressed in human myocardial tissues compared with other tissues. Combined with previous study [14] and this preliminary experiment, it was found that the expression of lnc-PXMP4-2-4 was highly expressed in H_2O_2 pre-treated cardiomyocytes compared with normal cardiomyocytes.

Janus kinase 2 signal transduction and transcriptional activator 3 (JAK2/STAT3) is the most well-studied signaling pathway in the JAK/STAT family [15]. Studies have shown that JAK2/STAT3 signaling pathway is enriched in AC16 injury model, which can eliminate mitochondrial ROS to protect cardiomyocytes from doxorubicin-induced damage [16] and JAK2/STAT3 signaling pathway is also involved in mediating protection of myocardium from I/R damage [17,18]. However, whether lnc-PXMP4-2-4 is involved in cardiomyocyte injury through activation of JAK2/STAT3 signaling pathway is still largely unknown, and it is a research direction to find out the relevant mechanism of action and eventually translate it into the clinic.

In this study, peripheral blood mononuclear cell (PBMC) samples from 24 patients with AMI on the day of admission, the first day after surgery, the third day after surgery and 24 patients with clinical control group were verified by PCR. Compared with the clinical control group, the expression of lnc-PXMP4-2-4 in PBMC of AMI patients was significantly higher than that of the control group. The expression of lnc-PXMP4-2-4 was significantly up-regulated on day 1 after PCI. In addition, in vitro cell experiments demonstrated that lnc-PXMP4-2-4 could further play a role in cell damage in acute myocardial infarction by activating JAK2/STAT3 signaling pathway. lnc-PXMP4-2-4 may be a potential target of acute myocardial infarction.

2. Materials and methods

2.1. Patient and sample selection

We collected 48 patients admitted to the emergency department of the Provincial Hospital Affiliated to Shandong First Medical University from September 2020 to November 2021, including 24 patients in the experimental group and 24 patients in the clinical control group. According to the 2015 ACC, AHA, and SCAI guidelines, the inclusion criteria were: (1) Clinical symptoms of chest pain. (2) Dynamic evolution of electrocardiogram. (3) There were changes in serum markers of myocardial necrosis. Inclusion criteria for clinical control group: Patients with chest pain symptoms, coronary artery CTA or coronary angiography showing that the degree of major vessel stenosis is less than 70%, or patients with mild calcification are referred to as coronary atherosclerosis patients, hereinafter referred to as clinical control group. The exclusion criteria for patients were: (1) Patients with severe arrhythmia, acute valvular insufficiency or acute heart failure due to other causes, as well as patients with chronic heart failure, pregnancy, cancer, acute infectious diseases, liver insufficiency and abnormal kidney function. (2) Family members and patients give up surgical treatment. All subjects settled in Shandong for a long time and signed informed consent. Our research protocol complies with the Declaration of Helsinki and has been approved by the Ethics Committee of Shandong First Medical University (Number: 2019-098, Date: November 13, 2019).

2.2. Culture and treatment of myocardial cell lines

Human cardiomyocyte line AC16 was purchased from Shanghai Zhongqiao Xinzhou Biotechnology Co. LTD. (Shanghai, China). The cells were supplemented with 10% of fetal bovine serum with high glucose DMEM (Sigma-Aldrich, St. Louis) (FBS; Excell, china) and supplemented with 100 U/mL penicillin and 100μg/mL streptomycin in a humid atmosphere containing 5% CO2 at 37 °C.

2.3. qRT-PCR assay

PBMC isolation: Peripheral blood mononuclear cells (PBMC: Lymphocyte and Monocyte) can be obtained by separating Human Peripheral Blood by Ficoll (IPHASE Human Peripheral Blood Monocyte Cells (Fresh) density gradient centrifugation method (2000 rpm, 20 min slow down setting no break). The obtained PBMC cells were stored in Trizol and then RNA was extracted.

Total RNA was extracted from PBMC and human myocardial cell lines using Trizol reagent (Vazyme, China) according to the instructions, and the RNA quantity was evaluated by NanoDrop 2000 (Thermofisher) to check the RNA concentration and purity. The cDNA was converted and stored in the refrigerator at −80 °C. The level of LncRNA was detected by real-qRT-PCR using SYBR Premix

Table 1
Small interfering RNA sequence.

Gene	Primer (5′→3′)
siRNA1	5′-GCCUCUCUCAUUCUUCAGUtt-3′
	3′-ACUGAAGAAUGAGAGAGGCtt-5′
siRNA2	5′-GUGGCCUCAUGUCAUUUCUtt-3′
	3′-AGAAAUGACAUGAGGCCACtt-5′
siRNA3	5′-CUGCCAUUAUUUCCUCUCUtt-3′
	3′-AGAGAGGAAAUAAUGGCAGtt-5′

ExTaq reverse transcription PCR kit (Accurate Biotechnology, Hunan, China). GAPDH as the internal parameter. The sequence of primers is shown in supplementary Table 1. Relative expression was detected using a standard protocol on the LightCycler® 480 assay system. The relative expression data were analyzed using formula $2^{-\Delta \Delta Ct}$. (Expression difference analysis was performed by GraphPad Prism 8).

2.4. Cell transfection

lnc-PXMP4-2-4 siRNA and negative control (NC) were purchased from GenePharma (shanghai, China), As shown in Table 1. The pcDNA 3.1 plasmid (pcDNA-PXMP4-2-4) with the complete lnc-PXMP4-2-4 sequence and the empty pcDNA-3.1 plasmid (pcDNA-Vector) were constructed by Jiman (Shanghai, China). pcDNA vectors were used as negative controls. The siRNA or plasmid is transfected into cells using the Lipofectamine 2000 reagent (Thermofisher) according to the manufacturer's protocol.

2.5. Cell viability assay

The CCK-8 Kit (BIOSS) measures cell viability by following these steps. Cardiomyocytes (5×10^3/well) were inoculated in 96-well plates, and the number of inoculated cells in each group was as equal as possible. After adhesion, the cells were treated with transfection reagents. Add 10 μL CCK-8 reagent to each well, mix with 90 μL medium and culture at 37 °C in an incubator away from light. After half an hour, the intensity of light absorption at 450 nm was measured using an enzyme-labeled instrument (ThermoMultiskan GO). The normalized OD ratio of experimental and control holes was used to calculate cell viability (cell viability = OD 450 nm experiment/OD 450 nm control).

2.6. Determination of lactate dehydrogenase

Lactate dehydrogenase (LDH) was measured using the LDH test kit (Solarbio, Beijing, China) according to the manufacturer's instructions. The absorbance of each hole was measured at 450 nm with an enzyme-labeled instrument (ThermoMultiskan GO).

### 2.7. Flow cytometry was used to detect cell mortality

For cell death analysis, cells were stained using the Annexin V-PE/7-AAD Apoptosis Kit (BD Pharmingen) and then the dead cells were detected by flow cytometry (Beckman FC400 MPL, USA). The interpretation of the dot plot of Annexin V-PE/7-AAD stained cells analyzed by flow cytometry varies from paper to paper.

2.8. Western blot analysis

Protein was extracted from AC16 cells by 100 μL containing 1 mM phenylmethyl sulfonyl fluoride (PMSF) and then isolated by 10% polyacrylamide gel electrophoresis (PAGE). And transfer it to a polyvinylidene fluoride (PVDF) film (Sigma-Aldrich Chemical Company, USA). Next, 5% skim milk was stirred and incubated at room temperature for 2 h, then the film was mixed with primary antibody (Bcl-2,ab194583; Bax,ab182733; JAK2,ab32101; STAT3,ab109085) or α-Tublin primary antibody (1:2000; 2125S, CST), etc. were treated overnight at 4 °C and then associated with secondary antibodies (1:2000; Santa Cruz Biotechnology, Dallas) incubated at room temperature for 2 h. Protein Western blot bands were detected using an electrochemical luminescence (ECL) kit (Biosharp, Shanghai, China) and Amersham Imager 600 Software.

2.9. Subcellular localization analysis

We predict the subcellular localization of key LncRNA by using LncLocator, a common platform based on stacked integrated classifiers. Used in LncLocator lncRNA subcellular localization information extracted from RNAlocate database (http://www.rnasociety.org/rnalocate), have empirical evidence to support. In addition, we used a fluorescence in situ hybridization kit from Ribo Bio (Guangzhou, China) to detect the cell localization of lnc-PXMP4-2-4 in AC16 cells according to the manufacturer's instructions, and the fluorescence signal was measured under a fluorescence microscope (Olympus BX51, Tokyo, Japan).
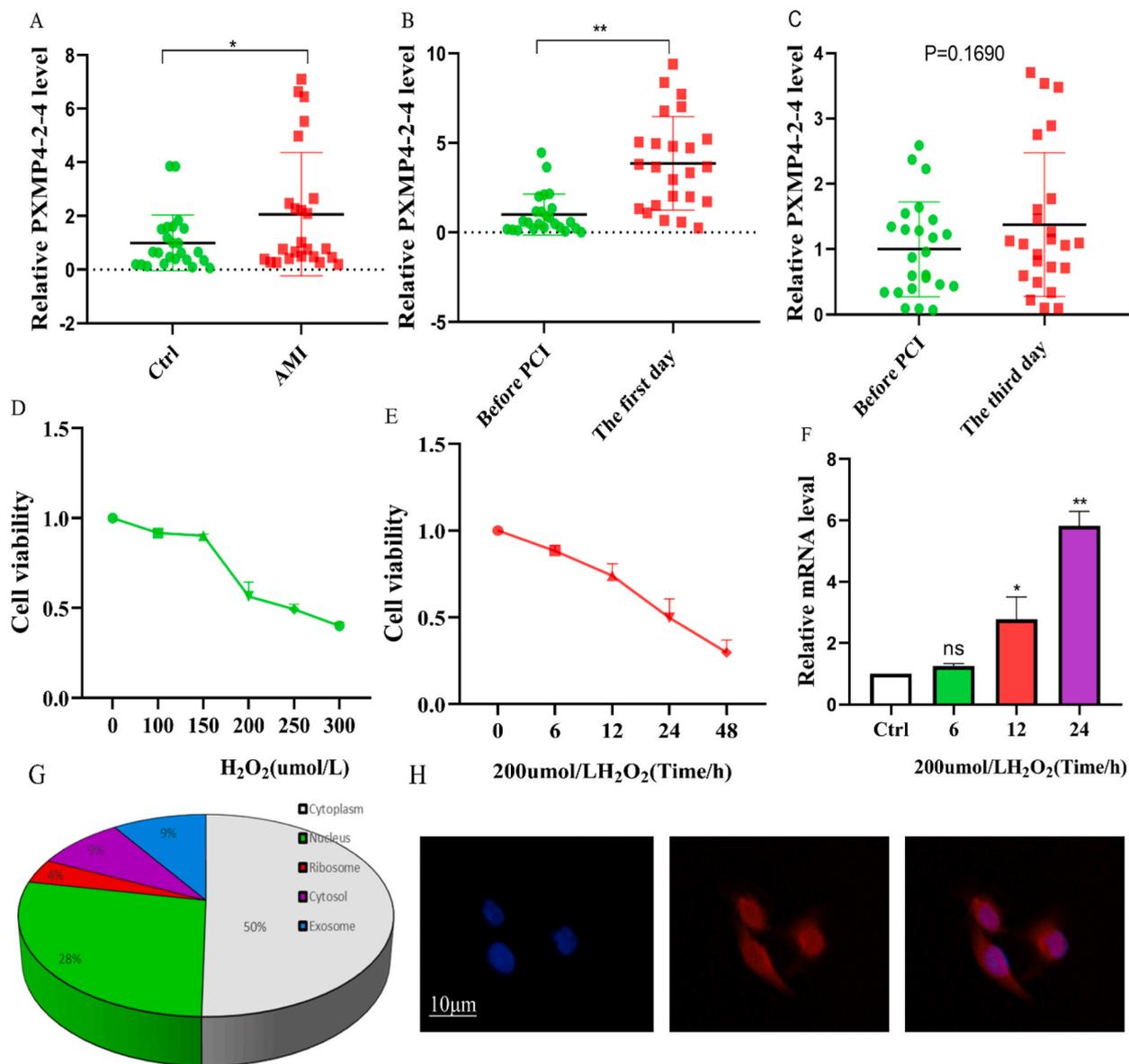
Fig. 1. Lnc-PXMP4-2-4 is upregulated in patients with coronary heart disease and myocardial cells. (A) qRT-PCR analysis of the expression level of lnc-PXMP4-2-4 in PBMCs of AMI patients with PCI indications compared with controls. (B) qRT-PCR analysis of the expression levels of lnc-PXMP4-2-4 in patients before PCI and the first day after PCI.(C) qRT-PCR analysis of the expression levels of lnc-PXMP4-2-4 in patients before PCI and the third day after PCI.N = 24, control (Ctrl) Significant differences compared to the corresponding control (D) The cell proliferation viability of AC16 cells under different concentrations of H_2O_2 was determined by CCK-8 analysis, and (E) the change curve of AC16 cell proliferation with H_2O_2 (200 µmol) treatment time. (F) qRT-PCR analysis of lnc-PXMP4-2-4 after 200 µmol H_2O_2 stimulation of AC16 cells for 0 h, 6 h, 12 h, and 24 h. (G) Site prediction of lnc-PXMP4-2-4 localized in the cytoplasm. (H)Using FISH to detect lnc-PXMP4-2-4 in AC16 cells. N = 3, control (Ctrl)significant differences compared to the corresponding control groups are shown as $*P < 0.05$ and $**P < 0.01$.

2.10. Statistical analysis

All experimental data are expressed as mean ± SD. Using GraphPad Prism 8, statistical significance was estimated for multi-group or two-group comparisons by ANOVA or Student t tests. $*P < 0.05$ is considered statistically significant.

Table 2
The correlation between lnc-PXMP4-2-4expression level andclinical characteristics of AMI.

Clinical parameters	lnc-PXMP4-2-4 expression		
	High expression (n = 12)	Low expression (n = 12)	P
Gender			
Male	5	7	0.342
Female	7	5	
Age			
>60 years old	6	5	0.500
≤60 years old	6	7	
Hypertension			
Yes	8	7	0.500
No	4	5	
Diabetes			
Yes	7	4	0.207
No	5	8	
Killip rating			
I-II	10	9	0.500
III-IV	2	3	
Ejection fraction			
<60	5	4	0.500
≥60	7	8	
Coronary stenosis			
≥90%	6	10	0.097
<90%	6	2	
Number of coronary stenosis			
1	6	7	0.500
>1	6	5	
Complete coronary occlusion			
Yes	1	4	0.158
No	11	8	

3. Results

3.1. Lnc-PXMP4-2-4 is up-regulated in patients with AMI

First, we performed qRT-PCR analysis on the PBMC of 24 AMI patients and 24 clinical control patients, and the results showed that the expression of lnc-PXMP4-2-4 in AMI patients was significantly up-regulated (Fig. 1A). In addition, qRT-PCR analysis was used to observe the changes of lnc-PXMP4-2-4, and it was found that the changes were significantly higher than those before surgery on the first day after surgery, and recovered to the preoperative level on the third day after surgery (Fig. 1B–C). The low or high expression of lnc-PXMP4-2-4 in peripheral blood mononuclear cells of AMI patients was classified according to the median value. High expression of lnc-PXMP4-2-4 was associated with degree of coronary artery stenosis (Table 2), but not with sex, age, hypertension, hyperlipidemia, Killip score, ejection fraction, and number of coronary artery stenosis.

In AC16, in order to further verify the modeling conditions, we conducted CCK-8 and PCR experiments for verification (Fig. 1D–F), and combined with literature, finally determined that 200 μmol H_2O_2 stimulation for 24 h was the optimal stimulation concentration and time. In addition, we used a stack-based integrated classifier to predict the subcellular localization of lncRNA (Fig. 1G). FISH experiment results showed that lnc-PXMP4-2-4 was mainly detected in the cytoplasm of AC16 (Fig. 1H).

3.2. Silence of lnc-PXMP4-2-4 aggravated the myocardial cell injury induced by H_2O_2

Next, Small interfering siRNA (Si-PXMP4-2-4) was used to down-regulate its expression. qRT-PCR showed that transfection of siRNA2 (si-2) in AC16 reduced lncPXMP4-2-4 levels by 80% (Fig. 2A). lncPXMP4-2-4 silencing decreased cardiomyocyte viability, promoted lactate dehydrogenase release, and increased cell death (Fig. 2B–E). Then, after silencing lncPXMP4-2-4, we treated AC16 with 200 μmol/L H_2O_2 for 24 h, and the results showed that silencing lncPXMP4-2-4 promoted H_2O_2-induced cell viability reduction, further increase of LDH release, and increase of cell death (Fig. 2F–I).

### 3.3. The overexpression of lnc-PXMP4-2-4 alleviates the H_2O_2-induced myocardial cell damage

Similarly, we constructed the lnc-PXMP4-2-4 overexpressed pcDNA-PXMP4 (UP) plasmid and used empty pcDNA3.1 (vector) as a control. After transfection of AC16 with pcDNA-PXMP4, the expression level of lnc-PXM-4-2-4 was increased by about 11 times (Fig. 3A). We found that cardiomyocytes transfected with UP had increased proliferation capacity, decreased LDH release, and decreased apoptosis compared with empty vectors (Fig. 3B–E). In addition, after 200 μmol/L H_2O_2 treatment of AC16 for 24 h, apoptosis was detected by CCK-8, LDH and flow cytometry, and it was found that lncPXMP4-2-4 overexpression alleviated H_2O_2-induced attenuation of AC16 cell viability, reduced LDH release, and inhibited apoptosis (Fig. 3F–I).
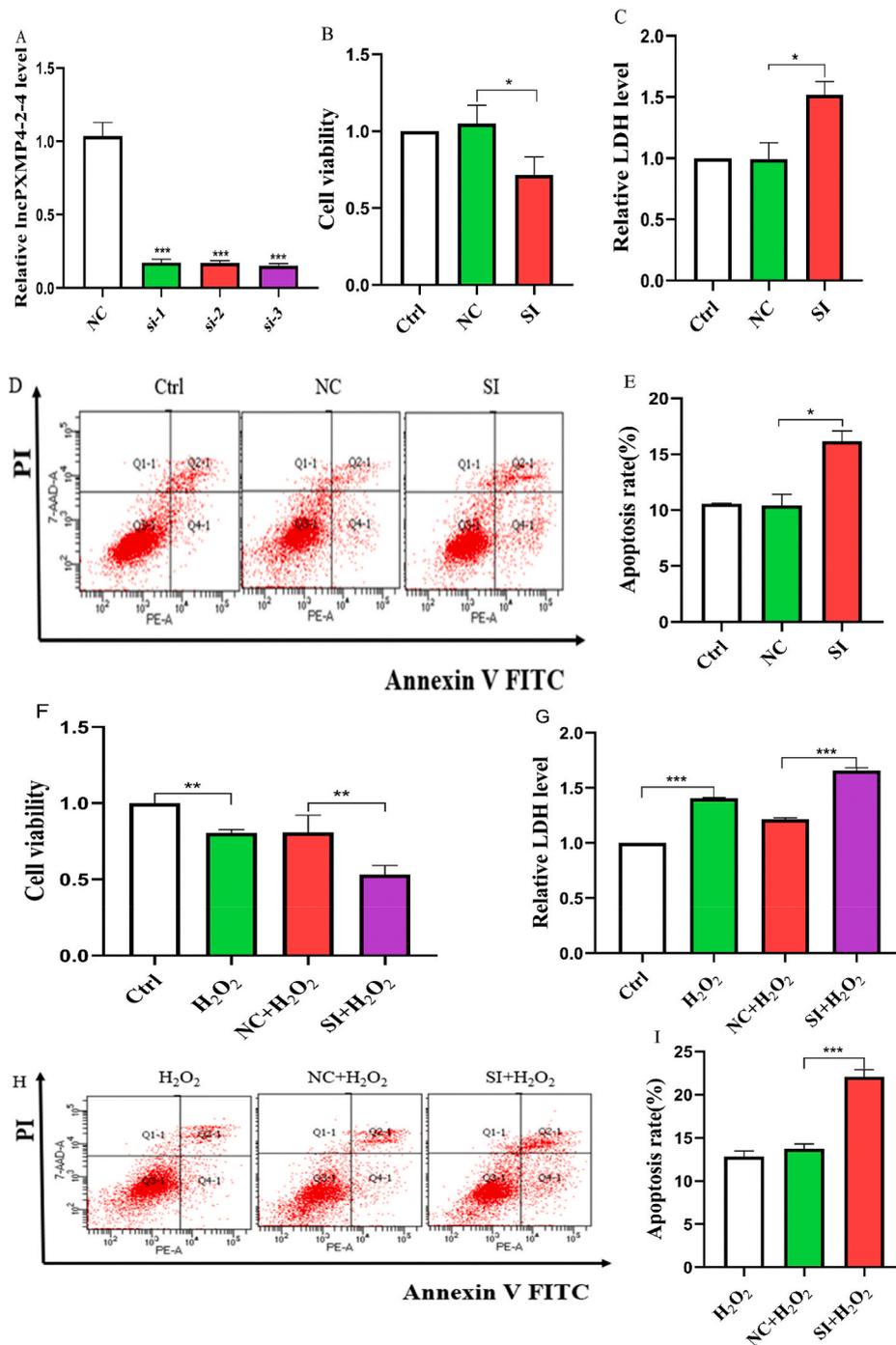
Fig. 2. Down-regulation of lnc-PXMP4-2-4 further aggravates H_2O_2-induced myocardial cell injury. (A) qRT-PCR analysis of the expression levels of lnc-PXMP4-2-4 in AC16 cells transfected with three different types of si-PXMP4-2-4 (si-1, si-2, si-3). (B) The proliferation of AC16 cells after transfection with si-NC (NC) or si-PXMP4-2-4 (SI) was detected by CCK-8 method. (C) LDH release in AC16 cells after NC or SI transfection. (D–E) Flow cytometry was used to detect the apoptosis rate of AC16 cells after transfection with si-NC (NC) or si-PXMP4-2-4 (SI). (F) The proliferation of AC16 cells treated with H_2O_2 after knockdown was determined by CCK-8 method; (G) the apoptosis of AC16 cells treated with H_2O_2 after knockdown was determined by LDH method; and (H–I) N = 3, control (Ctrl), significant differences compared to the corresponding control groups are shown as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.
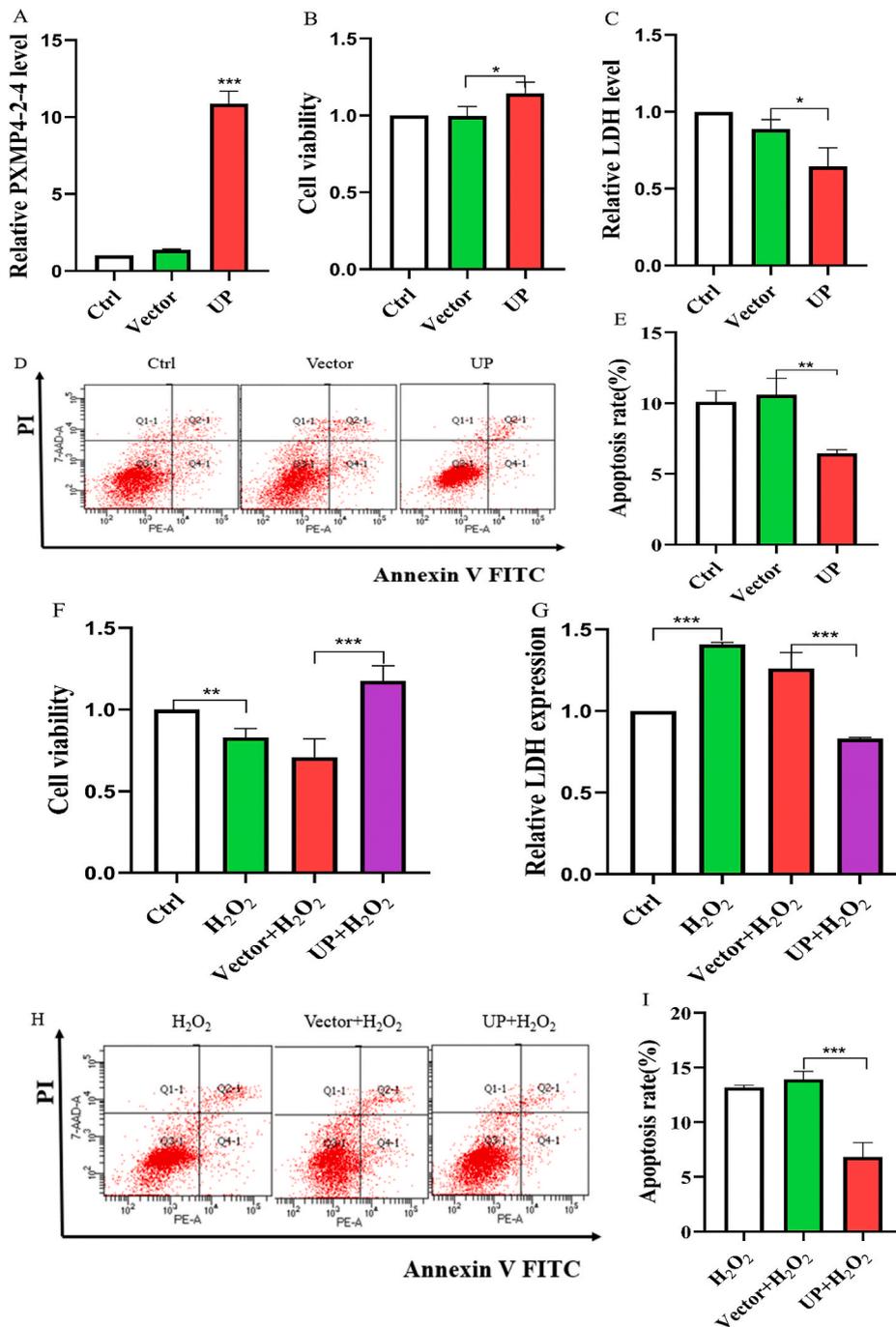
Fig. 3. Upregulation of lnc-PXMP4-2-4 attenuates H_2O_2-induced cardiomyocyte injury. (A) qRT-PCR analysis of lnc-PXMP4-2-4 expression levels in AC16 cells after transfection with pcDNA3.1 (Vector) or pcDNA-PXMP4 (UP). (B) The proliferation of AC16 cells after transfection with Vector or UP was detected by CCK-8 method. (C) Analysis of LDH expression in AC16 cells after overexpression. (D–E) Flow cytometry was used to detect the apoptosis rate of AC16 cells after overexpression. , (F) the proliferation activity of AC16 cells treated with H_2O_2 after overexpression was determined by CCK-8 method; (G) LDH expression was analyzed and the LDH release of AC16 cells treated with H_2O_2 after overexpression was analyzed; (H–I) the apoptosis rate of AC16 cells treated with H_2O_2 after overexpression was determined N = 3, control (Ctrl) significant differences compared to corresponding control groups are shown as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.
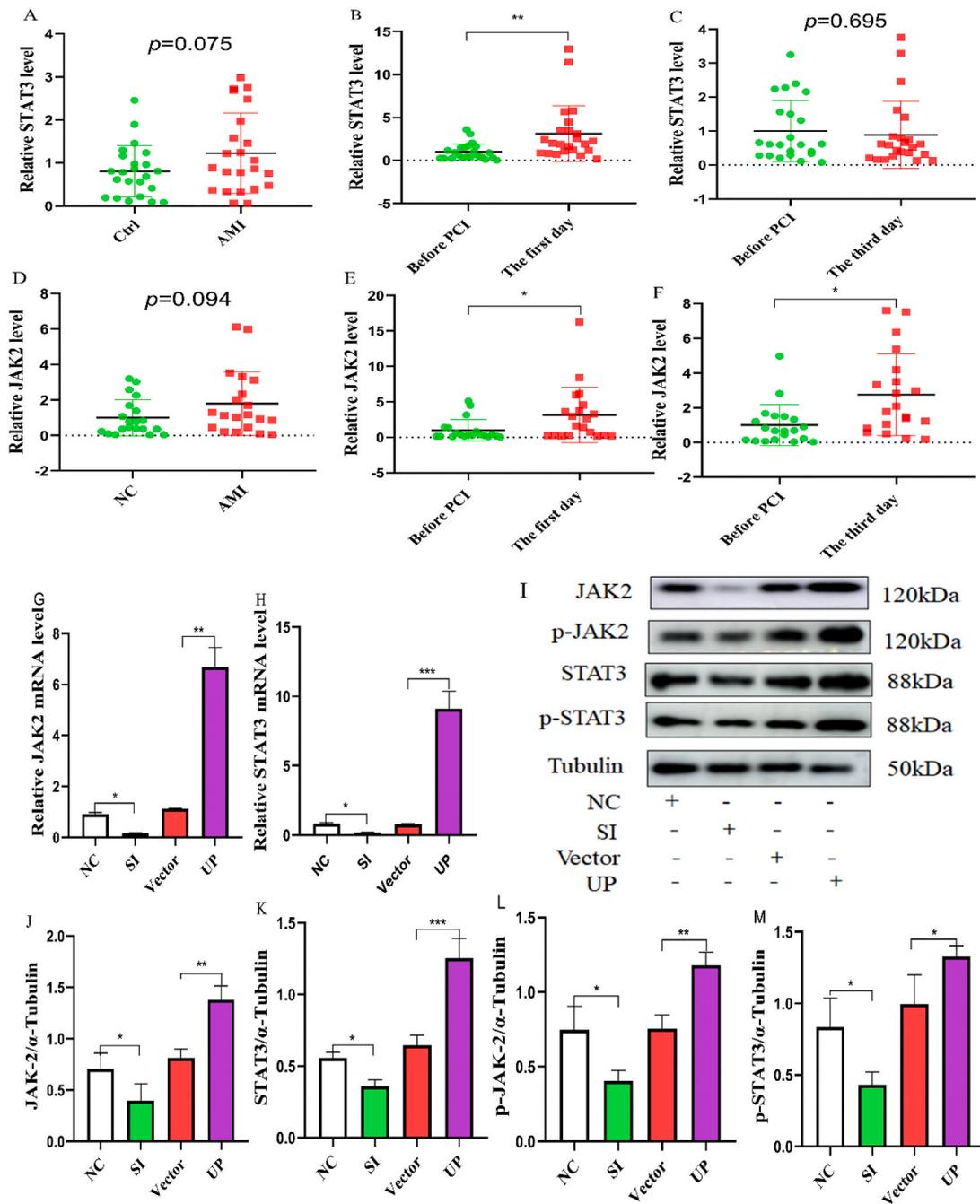
Fig. 4. LncPXMP4-2-4 activates the JAK2/STAT3 signaling pathway. A. qRT-PCR analysis of STAT3 gene expression level in AMI patients. (B) qRT-PCR analysis of STAT3 expression level on the first day after PCI compared with that before surgery (C) qRT-PCR analysis of STAT3 expression level before and on the third day after PCI (D) qRT-PCR analysis of JAK2 gene expression level in AMI patients (E) qRT-PCR analysis of JAK2 expression levels in patients before and on the first day after PCI. (F) PCR analysis of JAK2 expression levels in patients with AMI before and on the third day after PCI. N = 23 (21) (G) qRT-PCR analysis of JAK2 expression level after lncRNA knockdown or overexpression (H) qRT-PCR analysis of STAT3 expression level after lncRNA knockdown or overexpression (I)–(M) qRT-PCR analysis of JAK2 and STAT3 protein expression level after lncRNA knockdown or overexpression N = 3 *P < 0.05, **P < 0.01, and ***P < 0.001.
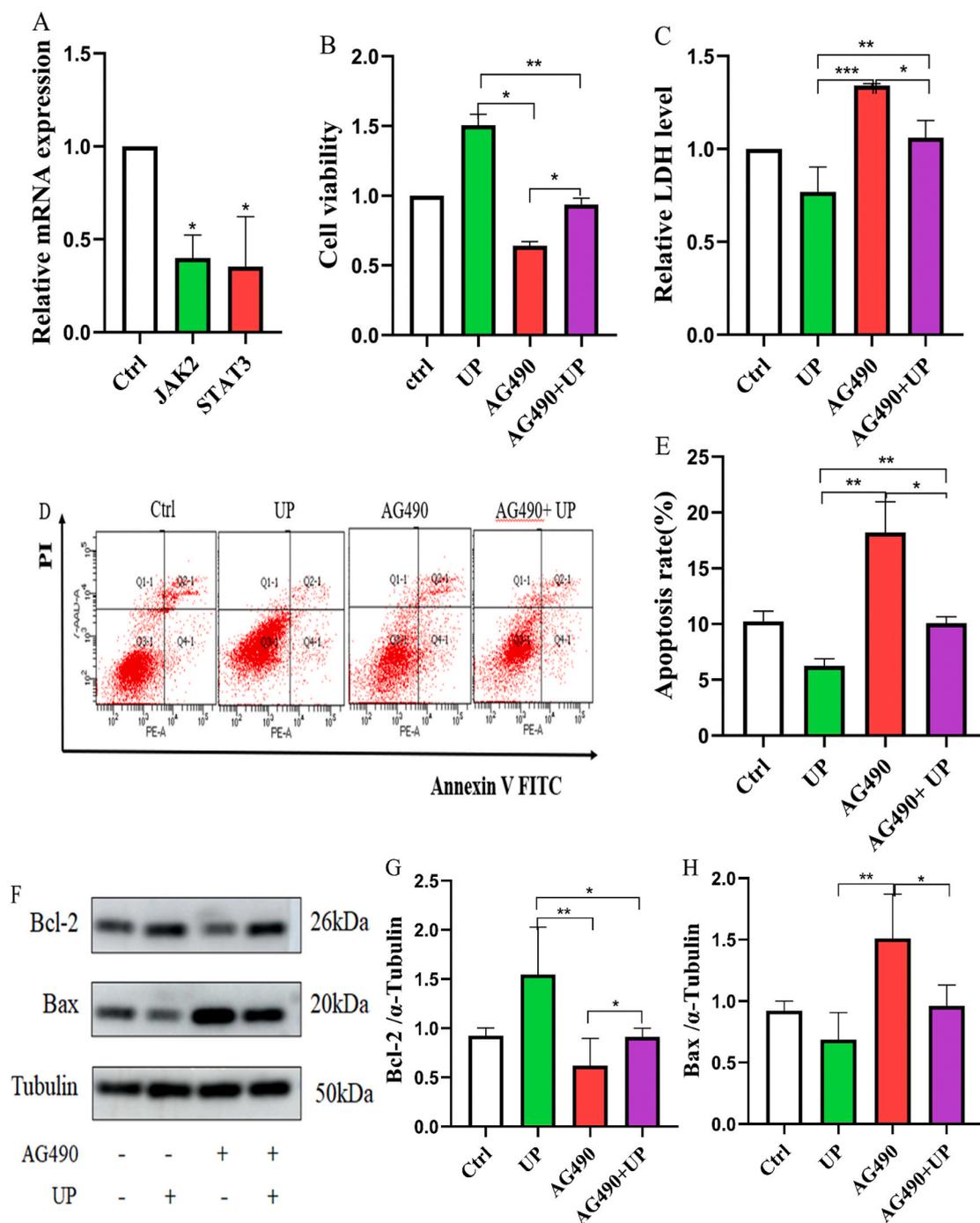
Fig. 5. AG490 eliminates the cardioprotective effects of lnc-PXMP4-2-4 (A) qRT-PCR analysis of STAT3 and JAK2 gene expression levels after AG490 inhibited JAK-STAT pathway. (B) Cell proliferation of AC16 cells co-transfected with AG490, pcDNA-PXMP4 (UP) or AG490+pcDNA-PXMP4 (AG490+UP) was determined by CCK-8; (C) LDH release levels of AC16 cells affected by AG490, UP or AG490+UP were determined by LDH. (D–E) Flow cytometry was used to analyze the apoptosis of AC16 cells affected by AG490, UP or AG490+UP, and (F–H) Western blot analysis was performed to analyze the expression of anti-apoptotic protein Bc-2 and pro-apoptotic protein Bax in AC16 cells affected by AG490, UP or AG490+UP.N = 3, significant differences compared to corresponding control groups are shown as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

3.4. Lnc-PXMP4-2-4 activates JAK2/STAT3 signaling pathway

PBMCS in blood samples from patients with acute myocardial infarction were collected on the day of admission, day 1 and day 3 after PCI, and changes in JAK2 and STAT3 were verified by qRT-PCR. Increased expression of STAT3 and JAK2 was found in patients before surgery (Fig. 4A and D). On the first day after surgery, the expressions of JAK2 and STAT3 were significantly higher than those before surgery (Fig. 4B and E). On the third day after surgery, the expression of STAT3 basically decreased to the preoperative level, while the expression of JAK2 was still significantly higher than the preoperative level (Fig. 4F and C). In addition, JAK2 and STAT3 mRNA and protein were down-regulated after lnc-PXMP4-2-4 silencing, and up-regulated after lnc-PXMP4-2-4 overexpression (Fig. 4G–M).

3.5. AG490 eliminates the cardioprotective effects of lnc-PXMP4-2-4

AG490 is a selective inhibitor of the JAK2/STAT3 signaling pathway, inhibiting the mRNA expression levels of JAK2 and STAT3 (Fig. 5A). Further experiments with CCK-8, LDH, and apoptosis measurements showed that AG490 partially inhibited JAK2/STAT3 and partially weakened the enhanced viability of AC16 cells, reduced LDH release, and decreased apoptosis induced by lnc-PXMP4-2-4 overexpression (Fig. 5B–E). Increased Bcl-2 expression and decreased Bax expression caused by decreased lnc-PXMP4-2-4 over-expression (Fig. 5F–H).

4. Discussion

It has been reported that lncRNAs are involved in the occurrence and development of cardiovascular diseases [19–23] For example, overexpression of LncRNA-CAIF can significantly inhibit H_2O_2-induced cardiomyocyte autophagy, and CAIF can further bind P53, thereby blocking cardiomyocyte death in ischemia-reperfusion injury [24,25]. Another study found that H19 expression was upregulated in acute myocardial infarction model mice, with increased apoptosis of cardiomyocytes after H19 knockout, while the opposite result was obtained after H19 overexpression [26]. Similarly, our experiment found that lnc-PXMP4-2-4 was up-regulated in AC16 cells stimulated by 200 μmol H_2O_2 for 24 h (Supplementary Figure 1A), and lnc-PXMP4-2-4 was taken as the research object. lnc-PXMP4-2-4 silencing inhibited JAK2/STAT3 signaling pathway, inhibited AC16 proliferation, promoted LDH release and increased apoptosis, and further inhibited cell viability during H_2O_2-induced myocardial cell injury. The up-regulation of lnc -PXMP-4-2-4 had the opposite effect.

In this study, we first demonstrated that the expression of lnc-PXMP4-2-4 in PBMC of AMI patients was significantly higher than that of the control group. Compared with pre-operation, the expression of lnc -PXMP4-2-4 was significantly up-regulated on day 1 after PCI, and recovered to pre-operation level on day 3 after surgery. It has been reported that within a few days after AMI, the number of peripheral blood monocytes and macrophages increases rapidly, and these initial infiltrating populations exhibit a pro-inflammatory phenotype, which changes to a major repair phenotype in the following days, coordinating the deposition of the scatricial tissue, and finally the PBMC returns to its original number level in the mature stage [5,27]. This was consistent with the change trend of PBMC in patients with lnc -PXMP4-2-4.

JAK2/STAT3 is involved in a variety of cardiovascular diseases [15,28,29]. JAK2/STAT has a protective effect on [30,31]. For example, JAK3/STAT3/Hexokinase II protects the heart from ischemia reperfusion (I/R) injuries [32]. AG490 protects brain ischemia/reperfusion injury by inhibiting JAK2/STAT3 signaling pathway [33]. Activation of JAK3/STAT3 signaling pathway can significantly up-regulate the synthesis of mitochondrial antioxidant enzymes, and reduce mitochondrial oxidative stress injury and myocardial injury [34]. We also observed that JAK2/STAT3 is involved in the regulation of cell damage caused by myocardial infarction. The expression of JAK2 and STAT3 in peripheral blood PBMC of clinical patients with AMI was increased before surgery, and the expression of JAK2 and STAT3 on the first day after surgery was significantly higher than before surgery. This is consistent with the change of lnc-PXMP-4-2-4 in peripheral blood. Therefore, we suspect that lnc-PXMP-4-2-4 may activate the JAK2/STAT3 signaling pathway. In AC16, JAK2/STAT3 was upregulated by qRT-PCR detection (Supplementary Figure 1B). Therefore, we observed the changes of JAK2 and STAT3 by down-typing and overexpression of lnc-PXMP4-2-4, and found that the mRNA expression of lnc -PXMP4-2-4 was positively correlated with JAK2 and STAT3. The inhibition of JAK2/STAT3 signaling pathway was further verified by AG490, a selective inhibitor of JAK2/STAT3 signaling pathway.

This study demonstrated for the first time that lnc-PXMP4-2-4 can activate the JAK2/STAT3 signaling pathway and play a protective role against H_2O_2-induced cardiomyocyte injury. However, there are several limitations to this study that need to be addressed. It should be confirmed by animal experiments that lnc-PXMP4-4 is upregulated in myocardial tissue of acute myocardial infarction. In addition, lnc-PXMP4-4 is a newly discovered lncRNA that may have other targets that need to be further explored in future studies.

5. Conclusion

Based on the above experimental results, we concluded that lnc-PXMP4-2-4 has a protective effect in cardiomyocytes and can activate the JAK2/STAT3 signaling pathway, which plays a protective role in acute myocardial infarction or H_2O_2 induced myocardial cell damage.

Author contribution statement

Hong Zhang: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Qingling Guo: Contributed reagents, materials, analysis tools or data; Wrote the paper. Guiju Feng: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data. Xin Shen, Xinxin Fen, Yi Guo, Shouyan Wang: Contributed reagents, materials, analysis tools or data. Xia Zhong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Data availability statement

The data used in this work is available in the supplementary material and will be made available upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviation list

LncRNAs   Long non-coding RNAs
AC16   Human cardiomyocytes
AMI   Acute Myocardial Infarction
CCK8   Cell Counting Kit
FISH   Fluorescence in situ hybridization
PBMC   Peripheral blood mononuclear cells
PCI   Percutaneous coronary intervention
LDH   Lactate dehydrogenase
CHD   Coronary heart disease
CVD   Cardiovascular disease
SI   Silencing lnc-PXMP4-2-4
pcDNA-PXMP4-2-4   Overexpression of lnc-PXMP4-2-4
pcDNA3.1   Empty vector
AG490   A selective inhibitor of the JAK2/STAT3 signaling pathway

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18649.

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