CELL CYCLE
2019, VOL. 18, NO. 23, 3393–3403
https://doi.org/10.1080/15384101.2019.1678965

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

LncRNA ANRIL knockdown relieves myocardial cell apoptosis in acute myocardial infarction by regulating IL-33/ST2

Jinhua Yang[a], Xianwei Huang[b], Fudong Hu[a], Xin Fu[a], Zhengming Jiang[a], and Kui Chen[a]

[a]Department of Cardiology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China; [b]Department of Emergency, the First Affiliated Hospital of Xiamen University, Xiamen, Fujian, China

ABSTRACT

Objective: To investigate the role of lncRNA ANRIL in the modulation of myocardial cell apoptosis in acute myocardial infarction (AMI).

Methods: AMI mice model was established, and lncRNA ANRIL, IL-33 and ST2 expressions were detected by quantitative real-time polymerase chain reaction (qRT-PCR) or western blot. The apoptosis of myocardial cells was detected by TUNEL assay. RNA pull-down and RNA immunoprecipitation (RIP) assays were used to confirm the interaction between lncRNA ANRIL and USP17.

Results: Compared with sham group, lncRNA ANRIL and ST2 expression levels were up-regulated, and the apoptosis of myocardial cells was increased in heart tissues of AMI group. Compared with normoxia group, the apoptosis of mouse myocardial cell HL-1 and primary murine myocardial cells was increased, and lncRNA ANRIL and ST2 expression levels were up-regulated in hypoxia group. We also found up-regulation of IL-33 in AMI group and hypoxia group. Besides, lncRNA ANRIL affected deubiquitinase USP17-mediated degradation of IL-33. Interfering lncRNA ANRIL reduced the apoptosis of myocardial cells through IL-33/ST2 pathway. *In vivo* experiments found that interfering lncRNA ANRIL relieved myocardial cell apoptosis and improved heart function in AMI mice.

Conclusion: LncRNA ANRIL regulated myocardial cell apoptosis through IL-33/ST2 pathway.

ARTICLE HISTORY
Received 28 February 2019
Revised 21 July 2019
Accepted 24 September 2019

KEYWORDS
LncRNA ANRIL; IL-33; ST2; acute myocardial infarction; apoptosis

Introduction

Acute myocardial infarction (AMI) is still a leading cause of morbidity and mortality worldwide, despite the improvements of therapeutic strategies over the past decade, which leads to angiostegnosis and vascular obstruction [1]. The common complications following AMI are stroke, pericarditis, left ventricular free wall rupture, and ventricular arrhythmias, etc [1–3]. Therefore, it is important to find effective strategies to prevent and treat AMI. Currently, studies have shown that preventing the apoptosis of myocardial cells can relieve hypoxia and ischemia-induced injury [4]. Our previous report also found that miR-128 modulated the apoptosis of myocardial cell line H9c2 to alleviate myocardial ischemia injury in AMI [5]. However, the molecular mechanism underlying myocardial cell apoptosis in AMI is still not fully understood.

Interleukin-33 (IL-33), an inflammatory cytokine that belongs to IL-1 cytokine family, plays a protective role in inhibiting inflammatory responses and myocardial apoptosis [6,7]. IL-33 also protects myocardial cells from doxorubicin-induced apoptosis through inhibiting ASK1/JNK signaling [8]. Moreover, IL-33 is the ligand for ST2, and IL-33/ST2 signaling pathway has been proved to be involved in the regulation of myocardial cell apoptosis. IL-33/ST2 signaling can prevent the apoptosis of myocardial cells, promote the survival of myocardial cells, reduce infarct size, fibrosis and apoptosis, and improve heart function after ischemia/reperfusion in rats [6]. Our previous report found that inhibition of IL-33 promoted the apoptosis of myocardial cells through up-regulating the expression of ST2 [5].

Long non-coding RNA antisense non-coding RNA at the INK4 locus (ANRIL), also known as lncRNA CDKN2B-AS1, is firstly found in patients with hereditary melanoma and neural system tumor [9].

CONTACT Jinhua Yang jinhua0371@163.com
Supplemental data for this article can be accessed here.

Studies have shown that lncRNA ANRIL is abnormally expressed in many cancers, such as hepatocellular carcinoma, esophageal squamous cell carcinoma (ESCC), cervical cancer, etc. [10,11]. Besides, the decrease of lncRNA ANRIL in intestinal mucosa is related to the increase of inflammation, severity and risk of Crohn's disease [12]. Importantly, lncRNA ANRIL is correlated with atherosclerotic cardiovascular disease by genome-wide association studies [13]. Moreover, lncRNA ANRIL is remarkably upregulated in left ventricle biopsies and peripheral blood monouclear cells of heart failure patients than control subjects [14], indicating that lncRNA ANRIL may be involved in the regulation of heart disease. Our preliminary experiment showed that lncRNA ANRIL was elevated in heart tissue of AMI rats than control rats. Thus, the present study aims to investigate whether lncRNA ANRIL promotes the apoptosis of myocardic cells and its underlying mechanism.

Materials and methods

Establishment of AMI mice model

Male C57BL/6 mice (weighing 25–27 g) were purchased from the laboratory animal center of Zhengzhou University, and kept in the cages with 12 h light/dark cycle under controlled temperature of 25°C with free access to food and water. All animal experiments were approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

The C57BL/6 mice were divided into two groups: namely the sham group and AMI group. All mice were intraperitoneally injected with 50 mg/kg pentobarbital sodium for anesthesia, then conducted left thoracotomy between the 3th and 4th intercostal space. Then, the cardiac tissue was exposed. In AMI group, 7/0 sterile suture was used to ligate left anterior descending artery (LAD). In sham group, the mice received similar surgical procedures but did not perform the ligation of LAD. Twenty-four hours after the surgery, echocardiographic parameters of all mice were assessed by 2D M-mode echocardiography and B-mode echocardiography. Heart tissues were collected and frozen with liquid nitrogen, then stored at −80°C for further use.

Ad-siRNA-ANRIL or Ad-NC adenovirus was purchased from GENECHEM (Shanghai, China). To determine the effect of interfering ANRIL on heart function, Ad-siRNA-ANRIL or Ad-NC adenovirus (5×10^9/100 μl) were injected into apex of heart of AMI mice. Seven days later, echocardiographic parameters of all mice were assessed by 2D M-mode echocardiography and B-mode echocardiography. Heart tissues were collected for the detection of ANRIL, IL-33 and ST2 expressions.

Cell culture and transfection

Mouse myocardial cell line HL-1 was purchased from American Type Culture Collection (ATCC, VA, USA) and cultured in modified Claycomb medium (Sigma, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 100 μg/ml penicillin–streptomycin (Gibco, USA), 0.1 mM norepinephrine (Sigma, USA) and 2 mM L-glutamine (Sigma, USA) at 37°C with 5% CO_2 and 95% air for 48 h. Then, the medium was changed to serum-free Claycomb medium.

Primary murine myocardial cell were isolated according to previous report [15]. Briefly, C57BL/6 mice were intraperitoneally injected with 50 mg/kg pentobarbital sodium for anesthesia. The heart was removed and conducted retrogradely aortic perfusion at constant pressure. When the heart became swollen and hard, the left ventricle was removed, cut into several pieces, and digested for 10 min at 37°C. The supernatant containing the dispersed myocytes was filtered into a sterilized tube and centrifuged at 500 rpm for 1 min. Primary murine myocardial cell were cultured in minimal essential medium (MEM; Gibco, USA) supplemented with 1.2 mM Ca^{2+}, 2.5% fetal bovine serum (Gibco, USA) and 1% penicillin–streptomycin (Gibco, USA). Small interfering RNA targeting ANRIL (si-ANRIL) and negative control (si-control), si-IL-33, ANRIL overexpressing plasmid (pcDNA-ANRIL) and negative control (pcDNA) were synthesized by GENECHEM (Shanghai, China), then transfected into the HL-1 cells using transfection reagent (Invitrogen, USA).

To establish hypoxia/reoxygenation *in vitro* model, HL-1 cells were exposed to 1% O_2/5% CO_2 for 2 h followed by reoxygenation for 30 min (hypoxia) or 21% O_2/5% CO_2 (normoxia).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from heart tissues or myocardial cells using TRIzol Reagent (Invitrogen, USA), and reversely transcribed to cDNA using PrimeScript One Step RT-PCR Kit (Takara, Japan). qRT-PCR was performed using One-Step TB Green PrimeScript RT-PCR Kit (Takara, Japan) in Applied Biosystems 7500 Real-Time PCR Systems (Applied Biosystems, USA). GAPDH was used as internal control. LncRNA ANRIL expression level was analyzed using $2^{-\Delta\Delta Ct}$ method.

Western blot

Total protein was extracted from heart tissues or myocardial cells using RIPA lysis and extraction buffer (Thermo Scientific, USA) with Halt protease inhibitor Cocktail (Thermo Scientific, USA) and Halt phosphatase inhibitor Cocktail (Thermo Scientific, USA). The concentration of protein was detected using Pierce BCA Protein Assay Kit (Thermo Scientific, USA). The proteins were separated using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane (Invitrogen, USA). The membrane was incubated with primary antibodies against IL-33 (1:1000; ab187060, Abcam), ST2 (1:2000; ab228543, Abcam), Bcl-2 (1:2000; ab196495, Abcam), Bax (1:1000; ab199677, Abcam), procaspase 3 (1:10,000; ab32499, Abcam), cleaved-caspase 3 (1:500; ab49822, Abcam) and GAPDH (1:10,000; ab181602, Abcam) at 4°C overnight and subsequently incubated with goat anti-rabbit IgG (HRP) for 1 h at 25°C. Western BLoT chemiluminescent substrate (Takara, Japan) was used to visualize the bands.

TUNEL assay

One Step TUNEL Apoptosis Assay Kit (Beyotime Biotechnology, China) was used to detect the apoptosis of myocardial cells. For heart tissue, 4% paraformaldehyde (Beyotime Biotechnology, China) was used to fix the frozen section. For HL-1 cells, 4% paraformaldehyde was used to fix the cells. Then, phosphate buffer solution (PBS) was used to wash the frozen section for two times. PBS containing 0.3% Triton X-100 was added to the frozen section or HL-1 cells. Finally, TUNEL solution (50 μl) was added for staining, and nuclear cells that labeled positively were considered as apoptotic cells. The positive cells in randomly six views were detected by a fluorescence microscope (Nikon, Japan). The ratio of apoptotic cells was calculated as positive cells/total cells.

RNA pull-down

RNA pull down assay was performed using Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Scientific, USA). The biotinylated ANRIL was labeled using Pierce RNA 3°C End Desthiobiotinylation kit (Thermo Scientific, USA). Proteins extracted from HL-1 cells were mixed with 50 pmol biotinylated ANRIL, and incubated with streptavidin magnetic beads (Thermo Scientific, USA). Western blotting was conducted to measure USP17 and IL-33 binding to the streptavidin-coupled beads.

RNA immunoprecipitation (RIP)

RIP assay was performed using Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, USA). HL-1 cells were collected by centrifugation at 200 × *g* for 5 min at 4°C. The supernatant was discarded, and cells (approximately 1×10^7) were re-suspended in 50 μl RIP lysis buffer supplemented with 0.125 μl RNase inhibitor and 0.25 μl protease K on the ice. The cell lysate in the tube was placed on the magnetic separator, and the supernatant was incubated with RIP wash buffer containing magnetic beads protein A/G conjugated with anti-USP17 or normal mouse IgG (negative control). RNA-binding protein complex was obtained, and ANRIL expression was detected in the precipitates.

Co-immunoprecipitation (Co-IP)

Co-IP assay was performed using Pierce Co-Immunoprecipitation Kit (Thermo Scientific, USA). HL-1 cells were transfected with pcDNA-ANRIL or pcDNA plasmid for 48 h, collected by centrifugation at 1000 × *g* for 5 min at 4°C. The

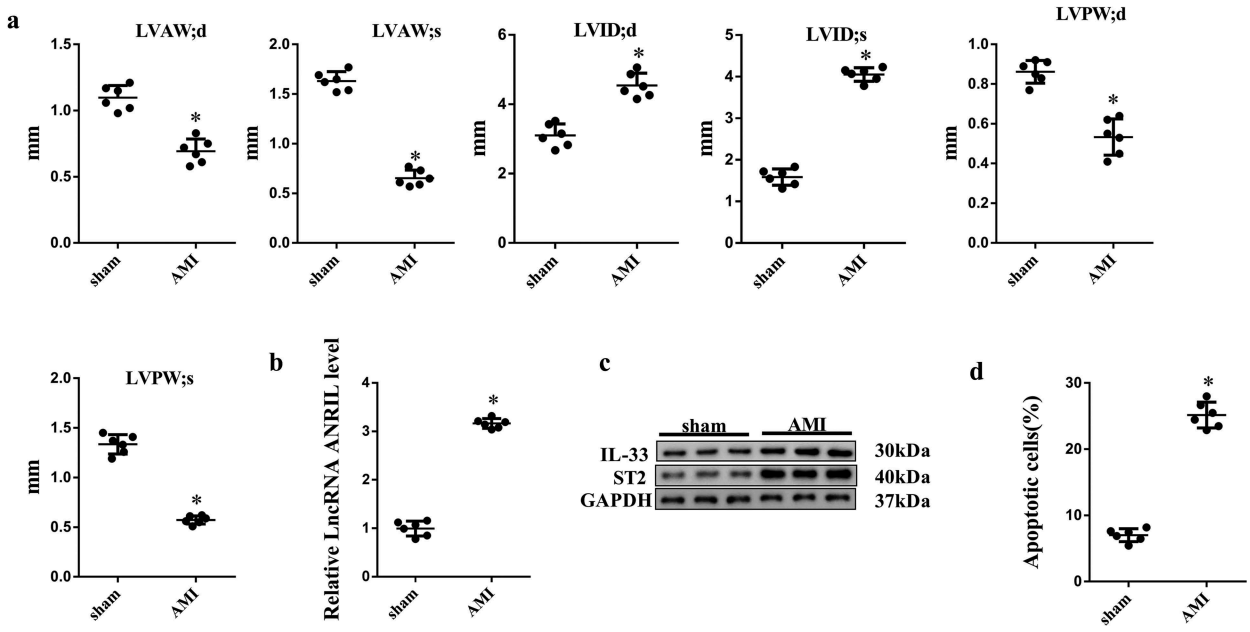

Figure 1. Overexpressed lncRNA ANRIL, IL-33 and ST2 in acute myocardial infarction (AMI) mice. C57BL/6 mice were divided into sham group (n = 6) and AMI group (n = 6). a. Echocardiographic parameters of left ventricular anterior wall thickness (LVAW), left ventricular internal diameter (LVID), left ventricular posterior wall thickness (LVPW) were assessed in sham and AMI groups 24 h after surgery. b. qRT-PCR was used to detect lncRNA ANRIL level in heart tissues of sham and AMI groups. c. Western blot was used to detect IL-33 and ST2 expression in heart tissues of sham and AMI groups. d. TUNEL assay was used to detect the apoptosis of myocardial cells in heart tissues of sham and AMI groups. *$p < 0.05$ vs sham. N = 6.

supernatant was discarded, and ice-cold IP Lysis/Wash Buffer was added to cells (10:1 v/w). Cell lysate was incubated on ice for 5 min, and centrifugated at 13,000 × g for 10 min at 4°C to remove cell debris. The supernatant was transferred to a new tube, and pre-cleared with 50 µl protein A agarose for 1 h at 4°C. Pre-cleared supernatants were incubated with anti-USP17 or normal mouse IgG at 4°C for 12 h. Control agarose resin slurry (40 µl) were added to each sample and incubated for 2 h at 4°C. Beads were pelleted, washed, and re-suspended in 200 µl of IP Lysis/Wash Buffer, and boiled for 5 min at 95°C. Western blot was used to detect USP17 and IL-33 in immunoprecipitated samples.

Statistical analysis

The data were normally distributed. Data were presented as mean ± SD, and were analyzed using Student's t-test or one-way analysis of variance (ANOVA) followed by the Newman-Keuls post hoc test for the comparisons between two groups or multiple groups, with $p < 0.05$ considered statistically significant.

Results

Overexpressed lncRNA ANRIL, IL-33 and ST2 in acute myocardial infarction (AMI) mice

To investigate the expression levels of lncRNA ANRIL, IL-33 and ST2 in heart tissues of AMI mice, AMI mice model was established by ligation of LAD. We found that echocardiographic parameters of left ventricular anterior wall thickness; diastole (LVAW; d), LVAW; systole (LVAW; s), left ventricular internal diameter; diastole (LVID; d), LVID; systole (LVID; s), left ventricular posterior wall thickness; diastole (LVPW; d) and LVPW; systole (LVPW; s) were significantly decreased, decreased, increased, increased, decreased and decreased in AMI groups than sham group 24 h after surgery (Figure 1a), which indicated that AMI mice were successfully established. Heart tissues were collected from mice of sham and AMI groups. As shown in Figure 1b, lncRNA ANRIL level was significantly up-regulated in heart tissues of AMI group than that of sham group. ST2 expression was significantly up-regulated in heart tissues of AMI group than that of sham group (Figure 1c). Moreover, we found up-regulation of

IL-33 in AMI group (Figure 1c). And the apoptosis of myocardial cells was significantly increased in heart tissues of AMI groups than that of sham group (Figure 1d).

Overexpressed lncRNA ANRIL, IL-33 and ST2 in myocardial cells exposed to hypoxia

Next, we established hypoxia/reoxygenation *in vitro* model to determine the expression levels of lncRNA ANRIL, IL-33 and ST2 in myocardial cells exposed to hypoxia. HL-1 and primary myocardial cells were exposed to normoxia or hypoxia. As shown in Figure 2a, the apoptosis of HL-1 and primary myocardial cells was significantly increased in hypoxia group than that of normoxia group. ST2 expression was significantly up-regulated in hypoxia group than that of normoxia group (Figure 2b). Moreover, we found up-regulation of IL-33 in hypoxia group (Figure 2b). Also, lncRNA ANRIL level was significantly up-regulated in hypoxia group than that of normoxia group (Figure 2c).

Interfering lncRNA ANRIL reduced the apoptosis of myocardial cells

To determine the role of lncRNA ANRIL in the apoptosis of myocardial cells *in vitro* hypoxia/reoxygenation model, HL-1 cells were transfected with si-ANRIL, then exposed to hypoxia condition. As shown in Figure 3a, lncRNA ANRIL

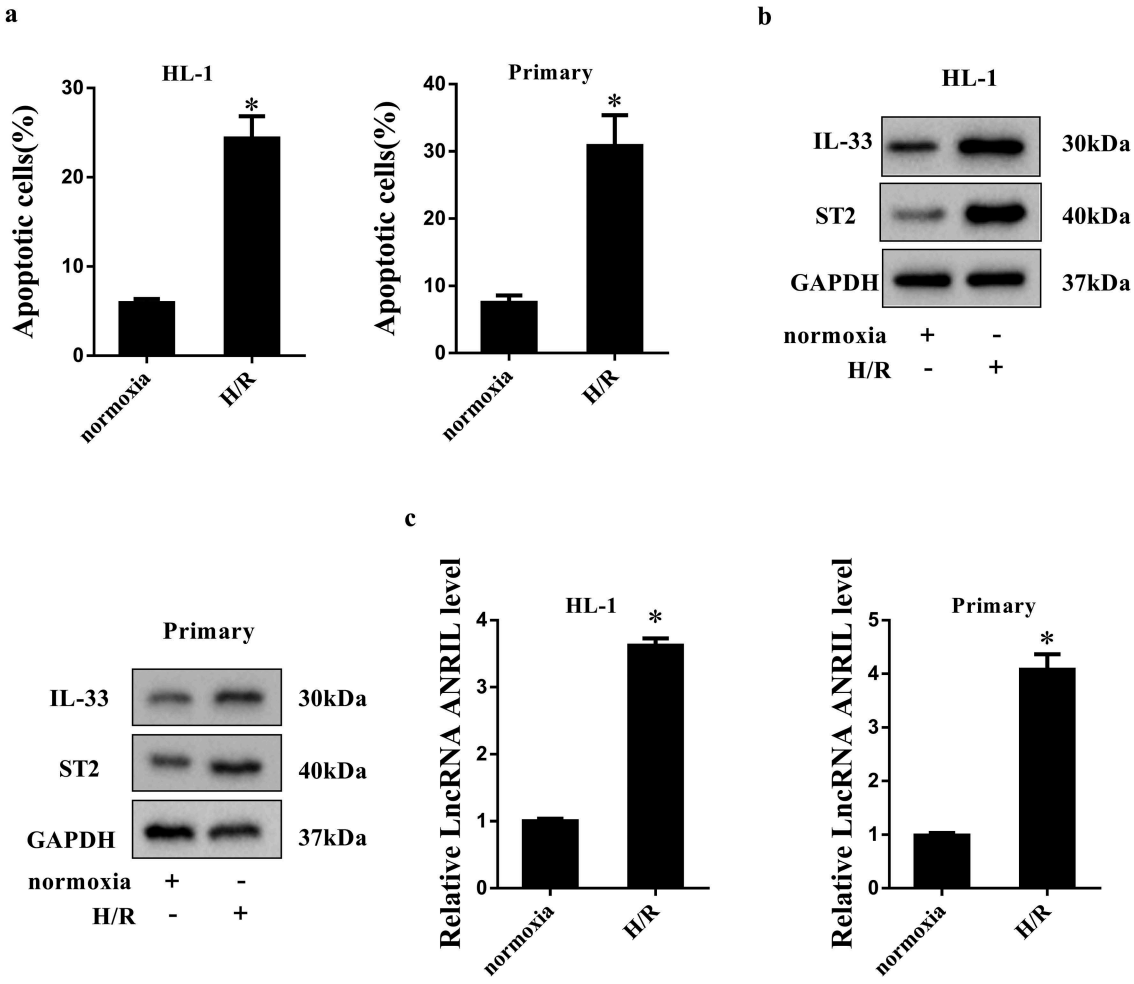

Figure 2. Overexpressed lncRNA ANRIL, IL-33 and ST2 in myocardial cells exposed to hypoxia. Mouse myocardial cells (HL-1) and primary myocardial cells were divided into normoxia and hypoxia groups. a. TUNEL assay was used to detect the apoptosis of HL-1 cells and primary myocardial cells in normoxia and hypoxia groups. b. Western blot was used to detect IL-33 and ST2 expression in normoxia and hypoxia groups. C. qRT-PCR was used to detect lncRNA ANRIL level in normoxia and hypoxia groups. *$p < 0.05$ vs normoxia. $N = 3$.

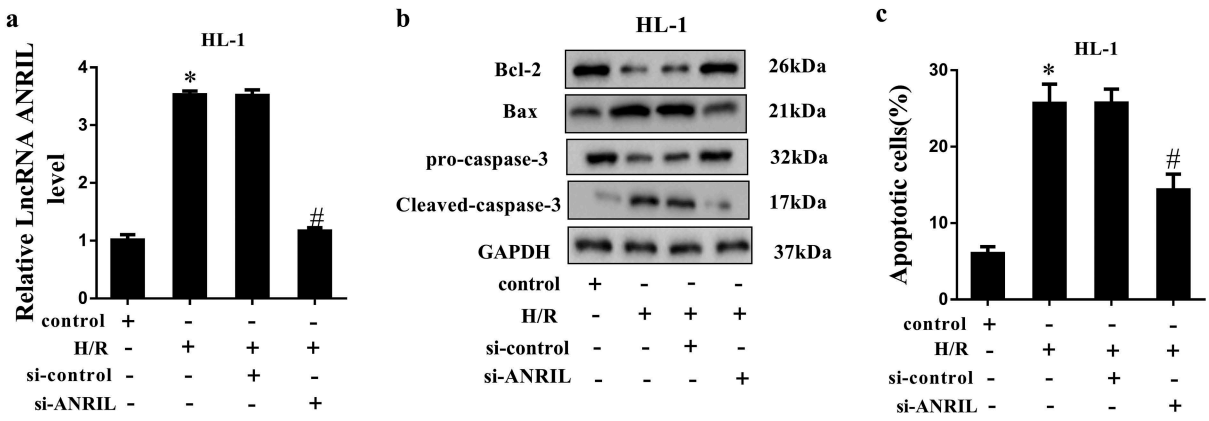

Figure 3. Interfering lncRNA ANRIL reduced the apoptosis of myocardial cells. HL-1 cells were transfected with si-ANRIL or si-control, then exposed to 1% O_2/5% CO_2 for 2 h followed by reoxygenation for 30 min (hypoxia) or 21% O_2/5% CO_2 (control). a. Compared with si-control group, lncRNA ANRIL was down-regulated in si-ANRIL group. b. Apoptosis related proteins Bcl-2, Bax, pro-caspase 3 and cleaved caspase 3 expressions were detected in si-control and si-ANRIL groups. c. Compared with si-control group, apoptosis of HL-1 cells was reduced in si-ANRIL group. $^{*}p < 0.05$ vs control; $^{\#}p < 0.05$ vs hypoxia + si-control. $N = 3$.

level was down-regulated in si-ANRIL group than that of si-control group. si-ANRIL promoted the anti-apoptosis protein Bcl-2, suppressed apoptosis protein Bax, increased pro-caspase 3 protein level and decreased cleaved-caspase 3 protein level (Figure 3b). In addition, si-ANRIL significantly suppressed the apoptosis of HL-1 cells (Figure 3c).

LncRNA ANRIL affected deubiquitinase USP17-mediated degradation of IL-33

It has been reported that enzyme ubiquitin-specific protease 17 (USP17) could affect IL-33 stability through deubiquitination of IL-33 [16], so we conducted RNA pull-down and RIP assays to determine whether lncRNA ANRIL could affect USP17-mediated degradation of IL-33. As shown in Figure 4a, USP17 and IL-33 expressions were detected in ANRIL pull-down complex. In addition, lncRNA ANRIL was significantly up-regulated in USP17 precipitate than IgG group (Figure 4b), indicating that ANRIL could bind to USP17. Next, we transfected pcDNA-ANRIL or pcDNA into HL-1 cells, and used USP17 antibody for immunoblotting (IgG used as control). We found pcDNA-ANRIL decreased the binding ability of USP17 and IL-33 (Figure 4c), indicating that ANRIL might affect IL-33 through regulating USP17. In HL-1 cells, IL-33 expression was down-regulated after ANRIL overexpression, and IL-33 expression was up-regulated after interfering ANRIL (Figure 4d). Moreover, ANRIL overexpression promoted the ubiquitination of IL-33 (Figure 4e). Under the treatement of CHX, pcDNA-ANRIL promoted the degradation of IL-33 with the time (Figure 4f). These findings suggested that lncRNA ANRIL could affect USP17-mediated degradation of IL-33.

Interfering lncRNA ANRIL reduced the apoptosis of myocardial cells through IL-33/ST2 pathway

As shown in Figure 5a, up-regulation of IL-33 was found in H/R group, and ST2 expression was up-regulated in H/R group. On that basis, further up-regulation of IL-33 could decrease ST2 expression to protect heart [17]. So, we transfected si-ANRIL into HL-1 cells, and found IL-33 expression was further up-regulated in H/R + si-ANRIL group, ST2 expression was decreased in H/R + si-ANRIL group, and si-IL-33 abolished the effect of si-ANRIL on IL-33 and ST2 expression (Figure 5a). The apoptosis of HL-1 cells was significantly increased in H/R group, and si-ANRIL further reduced the apoptosis of HL-1 cells, whereas si-IL-33 abolished the effect of si-ANRIL on the apoptosis of HL-1 cells (Figure 5b). Bcl-2 expression was down-regulated in H/R group, Bax expression was up-regulated in H/R group, pro-caspase 3 expression was down-regulated in H/R group, and cleaved-caspase 3 expression was up-regulated in H/R group. si-ANRIL up-regulated Bcl-2 expression, down-regulated Bax expression, increased pro-caspase 3 expression, and decreased cleaved-caspase 3 expression, whereas si-IL-33 abolished the effect of si-ANRIL on the expression of apoptosis-related proteins (Figure 5c).

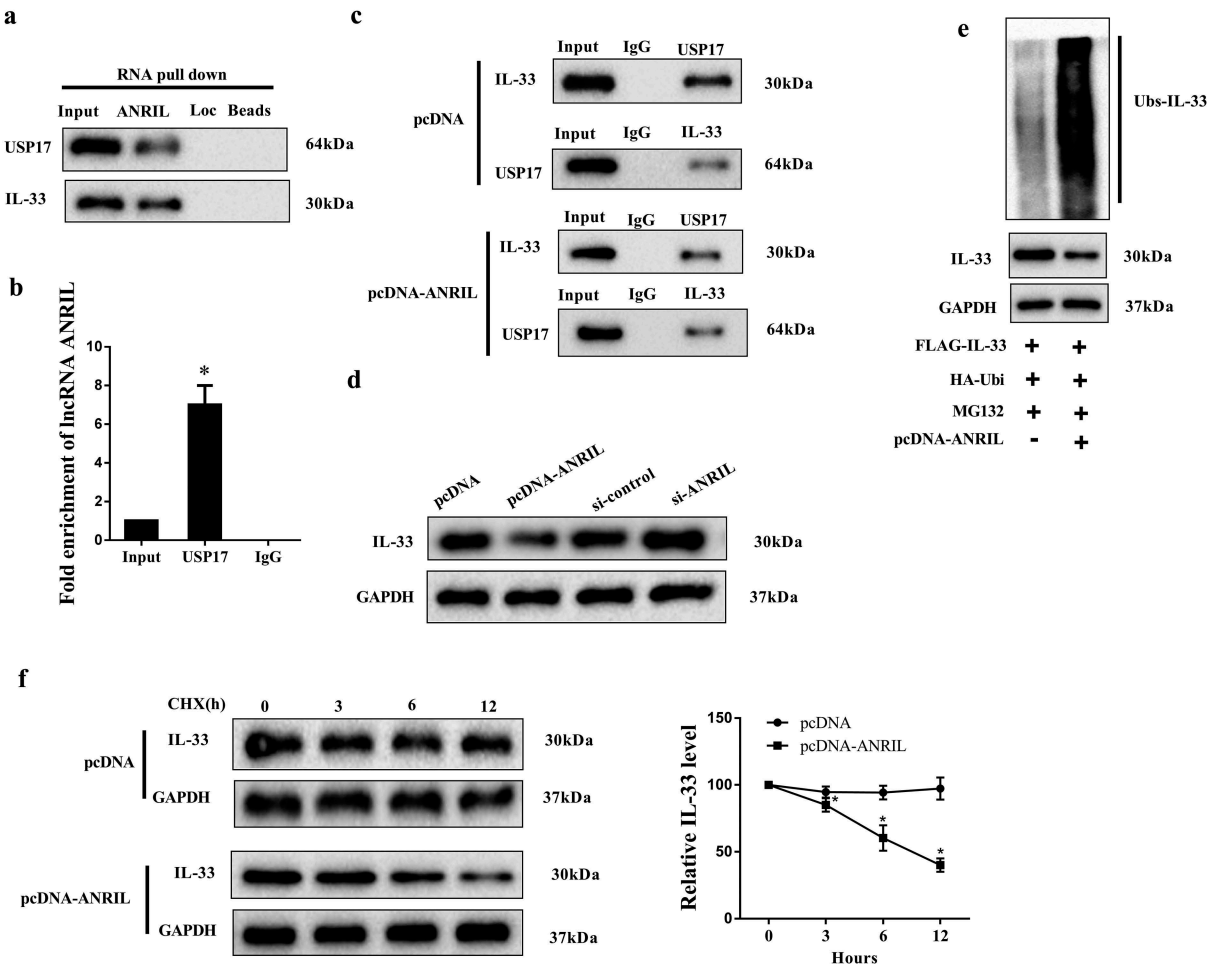

Figure 4. lncRNA ANRIL affected deubiquitinase USP17-mediated degradation of IL-33. a. RNA pull-down assay was used to detect USP17 and IL-33 expression in ANRIL pull-down complex. b. RIP assay was used to detect lncRNA ANRIL in USP17 precipitate. *$p < 0.05$ vs Loc or IgG. c. HL-1 cells were transfected with pcDNA-ANRIL or pcDNA, and USP17 antibody was used for immunoblotting (IgG used as control). Co-IP assay showed that lncRNA ANRIL overexpression decreased the binding ability of USP17 and IL-33. d. In HL-1 cells, IL-33 expression was down-regulated after ANRIL overexpression, and IL-33 expression was up-regulated after interfering ANRIL. e. ANRIL overexpression promoted the ubiquitination of IL-33. f. HL-1 cells were transfected with pcDNA-ANRIL or pcDNA, then treated with CHX (30 μg/ml) for 0 h, 3 h, 6 h and 12 h. pcDNA-ANRIL promoted the degradation of IL-33 with the time. *$p < 0.05$ vs pcDNA. $N = 3$.

Interfering lncRNA ANRIL relieved myocardial cell apoptosis in AMI mice

To verify interfering lncRNA ANRIL relieved myocardial cell apoptosis in AMI mice, Ad-siRNA-ANRIL or Ad-NC adenovirus was injected into apex of heart of AMI mice. Seven days later after adenovirus vector injection, we found echocardiographic parameters of LVAW; d, LVAW; s, LVID; d, LVID; s, LVPW; d, and LVPW; s were increased, increased, decreased, decreased, increased and increased in Ad-siRNA-ANRIL group than Ad-NC group, indicating that interfering lncRNA ANRIL improved heart function (Figure 6a). In addition, lncRNA ANRIL level was down-regulated in Ad-siRNA-ANRIL group than Ad-NC group (Figure 6b). IL-33 expression was up-regulated in Ad-siRNA-ANRIL group than Ad-NC group, whereas ST2 expression was down-regulated in Ad-siRNA-ANRIL group than Ad-NC group (Figure 6c). The apoptosis of myocardial cells in heart tissue was significantly reduced in Ad-siRNA-ANRIL group than Ad-NC group (Figure 6d).

Discussion

Increasing evidences show that lncRNAs play vital roles in regulating the apoptosis of myocardial cells in myocardial ischemia/reperfusion injury. For example, lncRNA MALAT1 was up-

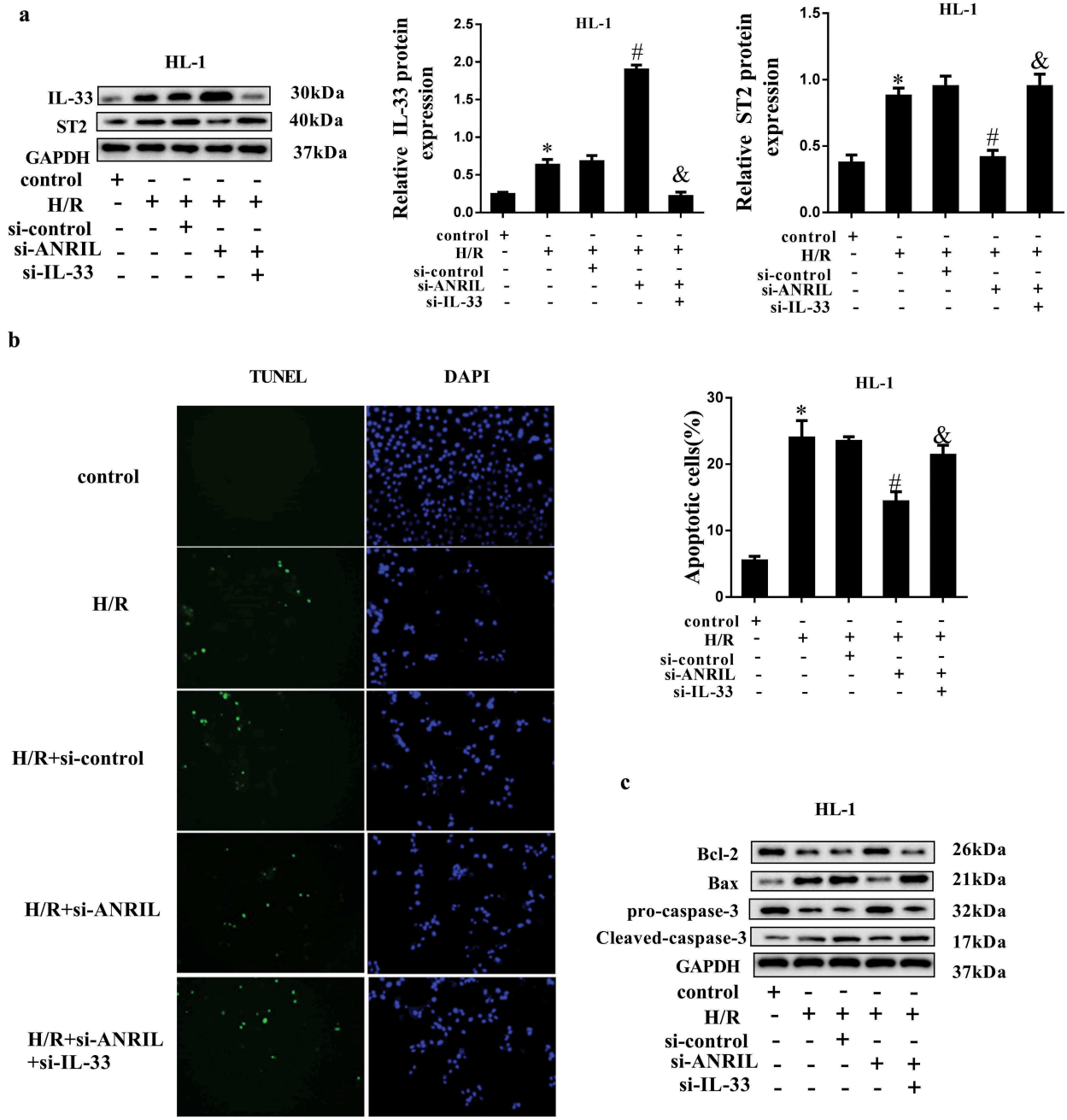

Figure 5. Interfering lncRNA ANRIL reduced the apoptosis of myocardial cells through IL-33/ST2 pathway. HL-1 cells were transfected with si-ANRIL or si-IL-33, then exposed to 1% O_2/5% CO_2 for 2 h followed by reoxygenation for 30 min (hypoxia) or 21% O_2/5% CO_2 (control). So, HL-1 cells were divided into control, H/R, H/R + si-control, H/R + si-ANRIL, H/R + si-ANRIL + si-IL-33 groups. a. Up-regulation of IL-33 was found in H/R group, and ST2 expression was up-regulated in H/R group. IL-33 expression was further up-regulated in H/R + si-ANRIL group, ST2 expression was decreased in H/R + si-ANRIL group, and si-IL-33 abolished the effect of si-ANRIL on IL-33 and ST2 expression. b. Bcl-2, Bax, pro-caspase 3, and cleaved caspase 3 expressions were detected in control, H/R, H/R + si-control, H/R + si-ANRIL, H/R + si-ANRIL + si-IL-33 groups. c. The apoptosis of HL-1 cells was detected in control, H/R, H/R + si-control, H/R + si-ANRIL, H/R + si-ANRIL + si-IL-33 groups. $N = 3$.

regulated in the infarction areas of patients with coronary heart disease, and MALAT1 reduced the isoproterenol-induced apoptosis of myocardial cells through miR-558/ULK1 signaling pathway to promote myocardial protection in myocardial infarction mice model [18]. LncRNA H19 was increased in myocardial cells of ischemia and reoxygenation (I/R) injury, and

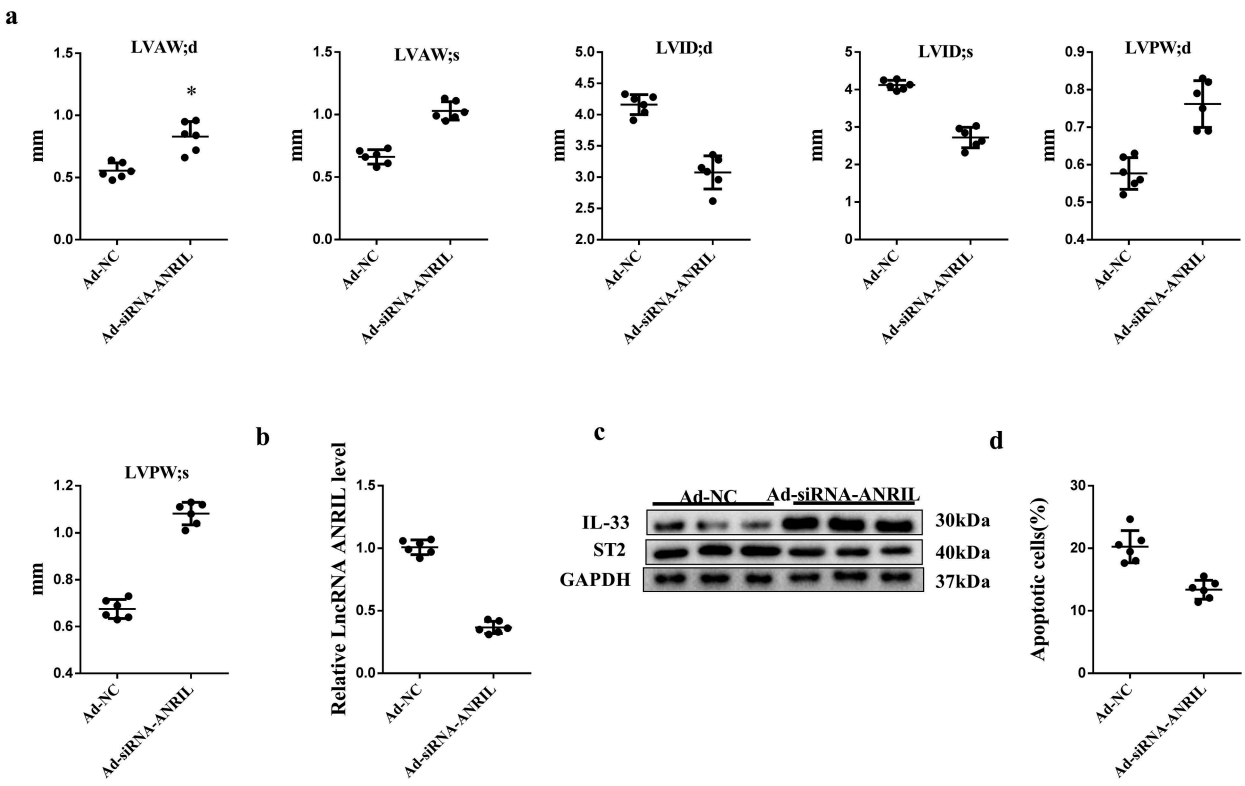

Figure 6. Interfering lncRNA ANRIL relieved myocardial cell apoptosis in AMI mice. AMI mice were established, and Ad-siRNA-ANRIL or Ad-NC (5×10^9/100 μl) adenovirus was injected into apex of heart. Seven days later, a. Echocardiographic parameters of LVAW, LVID and LVPW were assessed in Ad-NC and Ad-siRNA-ANRIL groups. b. LncRNA ANRIL level was detected in Ad-NC and Ad-siRNA-ANRIL groups. c. IL-33 and ST2 expressions were detected in Ad-NC and Ad-siRNA-ANRIL groups. d. The apoptosis of myocardial cells in heart tissue was detected in Ad-NC and Ad-siRNA-ANRIL groups. $N = 6$.

promoted the apoptosis of myocardial cells to aggravate myocardial I/R injury through miR-675/PPARα [19]. LncRNA TUG1 was elevated in rat myocardial infarction area, and knockdown of TUG1 increased the apoptosis of myocardial cells induced by hypoxia through miR-124/Hic-5 in myocardial infarction [20]. In this study, we found that lncRNA ANRIL was elevated in heart tissues in AMI mice and myocardial cells exposed to hypoxia, and interfering ANRIL reduced the apoptosis of myocardial cells, which indicated that the abnormal expression of lncRNA ANRIL was involved in the regulation of myocardial cell apoptosis in AMI. More studies on lncRNAs in the apoptosis of myocardial cells are needed to clarify the progression of AMI.

Studies have demonstrated that lncRNAs can regulate the progression of myocardial infarction through targeting miRNAs or modulate the ubiquitination-dependent degradation of proteins [21,22]. LncRNA autophagy-promoting factor (APF) modulated the autophagy of myocardial cells in myocardial infarction by directly binding and targeting miR-188-3p [21]. LncRNA AZIN2-sv involved in the regulation of angiogenesis and heart function after myocardial infarction through activating the ubiquitination-dependent degradation of talin1 and inhibiting phosphatase and tensin homologue/Akt pathway [22]. In this study, we confirmed that ANRIL could bind to USP17, and ANRIL overexpression decreased the binding ability of USP17 and IL-33. Besides, ANRIL overexpression promoted the ubiquitination of IL-33 and facilitated the degradation of IL-33 with the time under the treatement of CHX. Therefore, lncRNA ANRIL could affect USP17-mediated degradation of IL-33. In terms of novel aspects of this study, no other studies have investigated the role of lncRNA ANRIL in affecting the degradation of IL-33 in myocardial cells, which will enrich the literature and shed light on the underlying

mechanism of lncRNA ANRIL in myocardial cell apoptosis after AMI.

Numerous studies have shown that IL-33 could exert its functions by targeting ST2 in immune response or cancers [23,24]. IL-33 can recognize and interact with its specific receptor ST2 in immune cells, and anti-ST2 antibody reduced the effect of IL-33 on CD4 + T and CD8 + T cells during HIV-1 infection [23]. IL-33/ST2 axis was involved in the activity of mast cells during colitis, and IL-33 promoted the percentage of IL-13/IL-22 producing T cells, which indicated that IL-33 regulated T cell differentiation and function [24]. In addition, IL-33/ST2 axis was involved in the proliferation of ESCC cells and stromal cells, and the IL-33 or ST2 positive cells in ESCC stroma were positively related to node involvement, invasion depth and TNM stage [25]. Importantly, IL-33/ST2 axis was involved in the regulation of myocardial cell apoptosis [5,6]. In this study, we proved that the up-regulation of IL-33 protected the apoptosis of myocardial cells through negatively regulating ST2, which indicated that up-regulation of IL-33 occurred in myocardial cells in AMI. Moreover, we investigated the upstream molecules that modulate IL-33/ST2 in myocardial cell apoptosis after AMI, which is meaningful for the seek strategies for the prevention and treatment of AMI.

In conclusion, lncRNA ANRIL expression level was up-regulated in heart tissue of AMI mice and HL-1 cells exposed to hypoxia. Interfering lncRNA ANRIL reduced the apoptosis of myocardial cells through IL-33/ST2 pathway. This study demonstrated that lncRNA ANRIL served as an important molecular in the regulation of myocardial cell apoptosis through affecting IL-33 degradation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the Youth Innovation Fund Project of the First Affiliated Hospital of Zhengzhou University, Medical Science and Technology Project of Henan Health Department (No.201602050) and Foundation Project of Henan Education Department (No. 172102310263).

References

[1] Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. Lancet. 2017;389:197–210.
[2] Montone RA, Niccoli G, Minelli S, et al. Clinical outcome and correlates of coronary microvascular obstruction in latecomers after acute myocardial infarction. Int J Cardiol. 2017;236:30–35.
[3] Ying Ding W-LJ, Wen B, He Y, et al. A case of perinatal cardiomyopathy. Clin Surg Res Comm. 2019;3:21–25.
[4] Wu T, Wu D. Knockdown of long non-coding RNA-ZFAS1 protects cardiomyocytes against acute myocardial infarction via anti-apoptosis by regulating miR-150/CRP. J Cell Biochem. 2017;118:3281–3289.
[5] Yang J, Hu F, Fu X, et al. MiR-128/SOX7 alleviates myocardial ischemia injury by regulating IL-33/sST2 in acute myocardial infarction. Biol Chem. 2019;26;400 (4):533–544.
[6] Seki K, Sanada S, Kudinova AY, et al. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. Circ Heart Fail. 2009;2:684–691.
[7] Ruisong M, Xiaorong H, Gangying H, et al. The protective role of interleukin-33 in myocardial ischemia and reperfusion is associated with decreased HMGB1 expression and up-regulation of the p38 mapk signaling pathway. PloS One. 2015;10:e0143064–e0143064.
[8] Yao Y, Chen R, Ying C, et al. Interleukin-33 attenuates doxorubicin-induced cardiomyocyte apoptosis through suppression of ASK1/JNK signaling pathway. Biochem Biophys Res Commun. 2017;493:1288–1295.
[9] Pasmant E, Laurendeau I, Heron D, et al. Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. Cancer Res. 2007;67:3963–3969.
[10] Ma J, Li T, Han X, et al. Knockdown of LncRNA ANRIL suppresses cell proliferation, metastasis, and invasion via regulating miR-122-5p expression in hepatocellular carcinoma. J Cancer Res Clin Oncol. 2018;144:205–214.
[11] Zhang WY, Liu YJ, He Y, et al. Down-regulation of long non-coding RNA ANRIL inhibits the proliferation, migration and invasion of cervical cancer cells. Cancer Biomark. 2018;23:243–253.
[12] Ge Q, Dong Y, Lin G, et al. Long noncoding rna antisense noncoding RNA in the INK4 locus correlates with risk, severity, inflammation and infliximab efficacy in Crohn's disease. Am J Med Sci. 2019;357:134–142.
[13] Holdt LM, Teupser D. From genotype to phenotype in human atherosclerosis–recent findings. Curr Opin Lipidol. 2013;24:410–418.
[14] Greco S, Zaccagnini G, Perfetti A, et al. Long noncoding RNA dysregulation in ischemic heart failure. J Transl Med. 2016;14:183.

[15] Zhou -Y-Y, Wang S-Q, Zhu W-Z, et al. Culture and adenoviral infection of adult mouse cardiac myocytes: methods for cellular genetic physiology. Am J Physiol Heart Circ Physiol. 2000;279:H429–H436.
[16] Ni Y, Tao L, Chen C, et al. The deubiquitinase USP17 regulates the stability and nuclear function of IL-33. Int J Mol Sci. 2015;16:27956–27966.
[17] Wang EW, Jia XS, Ruan CW, et al. miR-487b mitigates chronic heart failure through inhibition of the IL-33/ST2 signaling pathway. Oncotarget. 2017;8:51688–51702.
[18] Guo X, Wu X, Han Y, et al. LncRNA MALAT1 protects cardiomyocytes from isoproterenol-induced apoptosis through sponging miR-558 to enhance ULK1-mediated protective autophagy. J Cell Physiol. 2018;234(7):10842–10854.
[19] Luo H, Wang J, Liu D, et al. The lncRNA H19/miR-675 axis regulates myocardial ischemic and reperfusion injury by targeting PPARα. Mol Immunol. 2019;105:46–54.
[20] Jiang N, Xia J, Jiang B, et al. TUG1 alleviates hypoxia injury by targeting miR-124 in H9c2 cells. Biomed Pharmacother. 2018;103:1669–1677.
[21] Wang K, Liu C-Y, Zhou L-Y, et al. APF lncRNA regulates autophagy and myocardial infarction by targeting miR-188-3p. Nat Commun. 2015;6:6779.
[22] Li X, Sun Y, Huang S, et al. Inhibition of AZIN2-sv induces neovascularization and improves prognosis after myocardial infarction by blocking ubiquitin-dependent talin1 degradation and activating the Akt pathway. EBioMedicine. 2019;39:69–82.
[23] Wu X, Li Y, Song C-B, et al. Increased expression of sST2 in early HIV infected patients attenuated the IL-33 Induced T cell responses. Front Immunol. 2018;9:2850.
[24] He Z, Song J, Hua J, et al. Mast cells are essential intermediaries in regulating IL-33/ST2 signaling for an immune network favorable to mucosal healing in experimentally inflamed colons. Cell Death Dis. 2018;9:1173.
[25] Cui G, Ren J, Xu G, et al. Cellular and clinicopathological features of the IL-33/ST2 axis in human esophageal squamous cell carcinomas. Cancer Cell Int. 2018;18:203.