H3K14 hyperacetylation-mediated c-Myc binding to the miR-30a-5p gene promoter under hypoxia postconditioning protects senescent cardiomyocytes from hypoxia/reoxygenation injury

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Abstract. Our previous study reported that microRNA (miR)-30a-5p upregulation under hypoxia postconditioning (HPostC) exert a protective effect on aged H9C2 cells against hypoxia/reoxygenation injury via DNA methyltransferase 3B-induced DNA hypomethylation at the miR-30a-5p gene promoter. This suggests that miR-30a-5p may be a potential preventative and therapeutic target for ischemic heart disease in aged myocardium. The present study aimed to investigate the underlying mechanisms of miR-30a-5p transcription in aged myocardium in ischemic heart disease. Cardiomyocytes were treated with 8 mg/ml D-galactose for 9 days, and then exposed to hypoxic conditions. Cell viability was determined using a cell viability assay. Expression levels of histone deacetylase 2 (HDAC2), LC3B-II/I, beclin-1 and p62 were detected via reverse transcription-quantitative PCR and western blotting. Chromatin immunoprecipitation-PCR and luciferase reporter assays were performed to evaluate the effect of c-Myc binding and activity on the miR-30a-5p promoter in senescent cardiomyocytes following HPostC. It was found that HPostC enhanced the acetylation levels of H3K14 at the miR-30a-5p gene promoter in senescent cardiomyocytes, which attributed to the decreased expression of HDAC2. In addition, c-Myc could positively regulate miR-30a-5p transcription to inhibit senescent cardiomyocyte autophagy. Mechanically, it was observed that increased H3K14 acetylation level exposed to romidepsin facilitated c-Myc binding to the miR-30a-5p gene promoter region, which led to the increased transcription of miR-30a-5p. Taken together, these results demonstrated that HDAC2-mediated H3K14 hyperacetylation promoted c-Myc binding to the miR-30a-5p gene promoter, which contributed to HPostC senescent cardioprotection.

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Introduction

As the global age increases, the morbidity and mortality resulting from myocardial infarction (MI) also increases each year (1). Although timely reperfusion can effectively reduce mortality, the recovery of blood flow through ischemic myocardium yields additional reperfusion injury, including myocardial stunning, reperfusion arrhythmia and myocardial necrosis (2). Myocardial ischemia/reperfusion (I/R) injury is affected by a variety of complex pathological mechanisms. For example, mitochondrial Ca^{2+} overload, platelet activation and micro-thrombosis formation, the disruption of mitochondrial membrane potential, free radical or reactive oxygen species and inflammatory responses (3). Among which, autophagy has also been found to be involved in I/R (4). Studies have reported that ischemic postconditioning (IPostC) can protect the heart from I/R injury by transient intermittent I/R episodes prior to long-term ischemia or hypoxia reperfusion (5). Wei *et al* (6) found that IPostC could improve autonomic function in acute ischemic stroke patients through the enhancement of the total autonomic nerve activity and vagus nerve activity. Another study also found that IPostC attenuates the injury in I/R myocardium by upregulating microRNA (miRNA/miR)-499 and inhibiting Toll-like receptor 2 activation (5). miR-30a-5p, a

member of the miR-30 family, is regarded as a key miRNA in cardiovascular pathophysiology (7). In addition, our previous study demonstrated that hypoxia postconditioning (HPostC) protected aged cardiomyocytes from hypoxia/reoxygenation (H/R) injury via DNA methyltransferase 3B (DNMT3B)-regulated miR-30a-5p, suggesting that miR-30a-5p may be a potential novel target for ischemic MI (8). However, the underlying mechanism involved in IPostC protection against aging myocardial I/R injury has not yet been elucidated.

Histone modification is a widely studied epigenetic modification, which has been demonstrated to result in the alteration of miRNA expression (9). As a type of histone modification, histone acetylation is generally correlated with transcriptional activation resulting from chromatin decondensation and thereby allowing transcriptional machinery access (10). This process is catalyzed by histone acetyltransferases (HATs) and histone deacetyltransferases (HDACs), which are responsible for adding and removing acetyl groups from histone tails, respectively. HDAC-mediated acetylation is associated with ischemic heart disease (11). Application of trichostatin A (TSA), a class I and class II HDAC inhibitor alters the response to myocardial ischemic injury in the heart and limited areas of MI (12). A study has shown that TSA can reduce post-ischemic infarct size, prevent myocardial remodeling after myocardial infarction, and protect myocardial function after myocardial I/R by inhibiting HDAC activity (13). Hence, histone acetylation plays a crucial role in ischemic heart disease, while its effect on aged heart I/R injury remains unknown.

Histone acetylation is essential for transcriptional regulation, which can change the affinity of transcription factors to DNA binding sites (14). As an important transcriptional factor, c-Myc regulates ~15% of genes in the genome, and can drive several biological processes, including cell apoptosis, proliferation, growth and differentiation (15). c-Myc widely participates in the development of disease via target genes (16). c-Myc can upregulate a series of transcriptional programs to directly regulate gene transcription (17). Additionally, c-Myc alters miRNA and long non-coding RNA expression patterns, which indirectly influences target gene expression in various diseases (18). For example, it has been reported that c-Myc functions as a modulator of polycystin-1 expression, likely via a feed-forward regulatory loop mechanism, in autosomal dominant polycystic kidney disease (19). Chen *et al* (20) found that vitamin D receptor signaling could regulate the c-Myc/Mad-1 network to inhibit the expression of the long non-coding RNA H19. Therefore, it is necessary to elucidate the relationship between histone acetylation and c-Myc responsible for miR-30a-5p transcription in senescent cardiomyocytes.

The present study revealed the role and epigenetic or transcriptional regulation mechanism of miR-30a-5p on the effects of HPostC in senescent cardiomyocytes. It was found that hyperacetylation of H3K14 promoted c-Myc binding to the miR-30a-5p gene promoter, which led to the increased transcription of miR-30a-5p and attenuated senescent cardiomyocyte I/R injury.

Materials and methods

Cell culture and treatment. H9C2 rat cardiomyocytes (The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences) were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin streptomycin in 5% CO_2 at 37˚C in a humified atmosphere. Senescent cardiomyocytes were established by 8 mg/ml D-galactose (Shanghai Bio-Tech Co., Ltd.) treatment of H9C2 cells for 9 days, which was based on findings from our previous study where increased senescence-associated β-galactosidase (SA-β-gal)-positive cells were identified using a Senescence β-Galactosidase staining kit (Beijing Solarbio Science & Technology Co., Ltd.) (8). Furthermore, H/R and HPostC models were established as previously reported (8). Senescent cardiomyocytes were treated with 200 nmol/l Trichostatin A (TSA; cat. no. HY-15144; MedChemExpress) or 1 μmol/l Romidepsin (cat. no. HY-15149; MedChemExpress) treatment for 24 h, and then H/R or HPostC treatment.

Cell adenovirus transduction. Adenovirus vectors ADV2 (U6/CMV-RFP) encoding Rattus HDAC2 and c-Myc-specific short hairpin RNA (shRNA) were obtained from Shanghai GenePharma Co., Ltd. The following sequences were included in the present study: Adenovirus sh-HDAC2 (Ad-shHDAC2), 5'-GGTATAGATGACGAGTCATAT-3'; Ad-shc-Myc, 5'-GAA TTTCTATCACCAGCAACA-3'; and Ad-sh negative control (NC), 5'-ACTACCGTTGTTATAGGTG-3'. The cytolysate products were centrifuged in a table centrifuge at 1,006.2 x g for 15 min at room temperature to harvest the viral supernatant. The AdEasy™ system (Shanghai GenePharma Co., Ltd.) was used for adenoviral vector construction. The pAdEasy (Stratagene; Agilent Technologies, Inc.) was used to package the plasmid, and the ratio of the ADV shuttle vector and packaging plasmid was 3~4:1. Subsequently, production and cell transduction of the adenovirus in 293T cells (The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences) was performed, and cells were flow-cytometrically sorted to maintain a GFP positivity rate >95%. Next, H9C2 cells cultured in a 25-cm^2 culture flask were incubated with Ad-shHDAC2, Ad-shc-Myc or Ad-shNC (the titer of the virus was $1x10^9$ PFU/ml) at a multiplicity of infection of 100. DMEM without FBS was used to dilute adenovirus. After incubation for 6 h, the medium was changed to serum-containing medium for 24-48 h, followed by treatments.

Cell viability assay. The cell viability of senescent cardiomyocytes was determined using a Cell Viability Imaging kit (cat. no. 06432379001; Merck KGaA) according to the manufacturer's protocols. Viable and dead cells are displayed as green and red fluorescence, respectively, under a confocal microscope (magnification, x10; Olympus FV1000; Olympus Corporation).

Reverse transcription-quantitative PCR (RT-qPCR). Rat cardiomyocyte RNA was extracted using RNAsimple Total RNA Kit (cat. no. DP419; Tiangen Biotech Co., Ltd.) and RNA was reverse transcribed into cDNA using a PrimeScript® RT reagent kit (Takara Bio, Inc.), according to the manufacturer's protocol. RT-qPCR was performed using SYBR Premix ExTaq™ (Takara Bio, Inc.) on the FTC 3,000 RT-qPCR System (Funglyn Biotech, Inc.). c-Myc, HDAC2 and β-actin primer sequences are listed in Table I, miR-30a-5p and U6

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	GenBank	Primer sequences (5'→3')
β-actin	NM_031144.3	F: TGTCACCAACTGGGACGATA
		R: GGGGTGTTGAAGGTCTCAAA
c-Myc	NM_012603.2	F: GCCTTTTCGTTGTTTTCCAA
		R: CACAGCAAACCTCCACACAG
HDAC2	NM_053447.1	F: GGCTGCTTCAACCTAACTG
		R: TTCACAATCAAGGGCAACTG

F, forward; R, reverse; HDAC2, histone deacetylase 2.

primers were purchased from Guangzhou RiboBio Co., Ltd. β-actin and U6 were used as internal reference genes. The 20 μl volume reaction system was comprised as follows: 10 μl SYBR Premix ExTaq™, 0.8 μl primers (10 μM), 2 μl cDNA and 6.4 μl ddH_2O. The following thermocycling conditions were used for qPCR: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 34 sec. Relative mRNA expression was calculated using the $2^{-\Delta\Delta Cq}$ method (21).

Western blotting. Cells were lysed in NP-40 buffer (cat. no. P0013F; Beyotime Institute of Biotechnology) containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40 and proteinase inhibitors. Protein concentration was determined using a BCA kit (cat. no. KGP902; Jiangsu KGI Biotechnology Co., Ltd.), according to the manufacturer's instructions. Total protein (30 μg) was separated via SDS-PAGE on 10% gel, and then separated proteins were transferred onto PVDF membranes (EMD Millipore). Following blocking with 5% non-fat milk for 2 h at room temperature, membranes were incubated at 4˚C overnight with LC3B-II/I (cat. no. ab192890; 1:1,000; Abcam), p62 (cat. no. ab56416; 1:1,000; Abcam), beclin-1 (BECN1; cat. no. ab210498; 1:1,000; Abcam), HDAC2 (cat. no. ab32117; 1:1,000; Abcam), c-Myc (cat. no. ab32072; 1:1,000; Abcam) and β-actin (cat. no. AC028; 1:10,000; ABclonal Biotech Co., Ltd.) antibodies. The membranes were then incubated with secondary antibodies (cat. no. M21002S; 1:5,000; Abmart Pharmaceutical Technology Co., Ltd.) for 1 h at 37˚C. Protein signals were visualized with enhanced chemiluminescence HRP substrate (cat. no. KF001; Affinity Biosciences) and semi-quantified using ImageJ software version 5.1 (National Institutes of Health).

Chromatin immunoprecipitation (ChIP)-PCR. An EZ-Magna ChIP™ kit (cat. no. 17-371; Sigma-Aldrich; Merck KGaA) was used for ChIP. Chromatin was immunoprecipitated for 24 h at 4˚C using anti-HDAC2 (cat. no. ab124974; 1 μg; Abcam), anti-H3K14ac (cat. no. ab203952; 1 μg; Abcam) and anti-c-Myc (cat. no. 18583S; 1 μg; Cell Signaling Technology, Inc.) antibody. Rabbit IgG immunoprecipitation was used as a negative control. 1/100 of total cell lysate was used as an internal control. qPCR was used to analyze precipitated DNA using specific primers (Table II) and it was performed as described above. The signals were calculated as the percentage of input.

Construction of deletion and site-directed mutagenesis of the miR-30a gene promoter. miR-30a-5p gene promoter sequence analysis, reporter gene construction and promoter activity study miR-30a-5p gene promoter sequences (2,200 bp) were downloaded from the University of California Santa Cruz Genome Browser Database (http://genome.ucsc.edu/). The c-Myc binding sites in the promotor of the miR-30a-5p gene were analyzed using the JASPAR database (http://jaspar.genereg.net/). The truncated vectors PGL3-rno-miR-30a-5p-F1-mut1 (5'-GTGACGACCAGTGTGGACCT-3'), PGL3-rno-miR-30a-5p-F1-mut2 (5'-GTGTGGACCTTTGTACATGG-3') and PGL3-rno-miR-30a-5p-F1-mut3 (5'-GTGACGACCAGTGTGGACCTTTGTACATGG-3') were chemically synthesized, and *Nhe*I/*Xho*I restriction sites were added to both ends of the target fragment during synthesis. The synthesized gene fragments were cloned into the PUC57 vector (Shanghai YingBiotech Company) and fused to the firefly luciferase reporter vector pGL3-basic (Shanghai YingBiotech Company).

Dual-luciferase assay. The core promoter sequence of miR-30a-5p was cloned into the PUC57 vector to construct a luciferase reporter plasmid, which had a c-Myc binding site at 700 bp upstream of the transcription start site of miR-30a-5p. Then, the full-length sequence of c-Myc was co-transfected with the full-length sequence of miR-30a-5p gene promoter region (wild-type, -700/+1, pGL3-P1) and the three full-length sequences of miR-30a-5p gene promoter region with c-Myc binding site mutation (mutant-type, pGL3-MT1, pGL3-MT2, pGL3-MT3) to detect the effect of c-Myc on the transcriptional activity of miR-30a-5p gene promoter region. 293T cells were seeded at 8×10^4 cells per well in 24-well plates, and 24 h after plating, the cells were transfected using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. In each well, 100 ng wild-type or mutant-type plasmid RNA vector and *Renilla* luciferase were co-transfected. The former culture medium was discarded 48 h after transfection, and the cells were then rinsed twice with PBS. Then, 100 μl passive lysis buffer was added in each well with cells and slightly shaken at room temperature for 15 min, followed by collection of the cell lysate. The program was set for 2 sec for pre-reading and 10 sec for value reading, with 100 μl Stop & Glo® Reagent (Promega Corporation) added for each sampling session. Then, prepared Stop & Glo Reagent and the luminescent plate or tube containing the cell lysate (20 μl/per sample) were placed into a bioluminescence detector. Finally, the program was operated and data were recorded after fluorescence reading. The *Renilla* luciferase

Table II. Primer sequences used for chromatin immunoprecipitation-PCR assay.

Gene	Primer sequence	Length (bp)
miR-30a-5p	F: ATGTTGTAGTCCTAGTAAGTCACCT	25
	R: TCTGTAAACTGTAAAGCCTCGT	22

F, forward; R, reverse; miR, microRNA.

signal was normalized to the firefly luciferase signal. This process was performed in triplicate for each target vector.

Database search. HDAC2 was found to regulate histone deacetylation under HPostC treatment, as predicted by Uniprot (http://www.uniprot.org/). First, HDAC2 (accession no. F7ENH8_RAT) was searched on the Uniprot website in the UniProtKB field to query the protein function. Bioinformatics analysis of HDAC2 in the 'Biological process' category of Gene Ontology (GO) showed the association of HDAC2 with 'histone H3 deacetylation' (red underline), especially for 'histone deacetylase activity (H3-K14 specific)' (red underline GO ID:0031078). The Uniprot database was searched in order to identify the known GO terms associated with HDAC. However, this was not the same as performing bioinformatics analysis (GO enrichment analysis) (22).

Statistical analysis. All data were collected and analyzed with GraphPad Prism 6.0 (GraphPad Software, Inc.). Data are expressed as the mean ± SD. All experiments were conducted three times. Differences between two groups were evaluated using an unpaired Student's t-test. Differences between no more than three groups were compared using one-way ANOVA followed by Student-Newman-Keul's test. Differences between four or more groups were analyzed using ANOVA followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

HDAC2-mediated H3K14 hyperacetylation in senescent cardiomyocytes under HPostC treatment. Histone acetylation plays a vital role in the regulation of gene expression (23). To investigate whether miR-30a-5p upregulation was affected by histone modification in senescent cardiomyocytes under HPostC treatment, TSA, a HDAC inhibitor, was applied to suppress HDAC enzyme activity. As shown in Fig. 1A, TSA significantly enhanced miR-30a-5p expression, which implied that histone acetylation participated in miR-30a-5p transcription under HPostC treatment. In addition, ChIP-PCR was used to detect H3K14 acetylation levels of the miR-30a-5p promoter in senescent cardiomyocytes. The results showed that, compared with the normoxia conditions, H/R significantly decreased the levels of H3K14ac at the miR-30a-5p gene promoter in senescent cardiomyocytes, whereas HPostC led to increased H3K14ac levels in senescent cardiomyocytes compared with H/R (Fig. 1B and C).

To determine the cause of the hyperacetylation at the miR-30a-5p gene promoter under HPostC treatment, the functions of HDAC2 was predicted by searching Uniprot. The Uniprot website showed that under the GO 'Biological process' category HDAC2 was found to be associated with 'histone H3 deacetylation' (underlined in red), specifically 'histone deacetylase activity (H3-K14 specific)' (underlined in red, GO ID:0031078) (Fig. S1). RT-qPCR and western blotting results showed a significant decrease in HDAC2 expression in senescent cardiomyocytes exposed to HPostC compared with the H/R group (Fig. 1D and E). Next, ChIP-PCR was performed to determine whether HDAC2 directly binds to the coding sequence region of miR-30a-5p. Cross-linked chromatin samples were extracted from the senescent cardiomyocytes and precipitated with an anti-HDAC2 antibody. As presented in Fig. 1F and G, HPostC inhibited the binding of HDAC2 to the miR-30a-5p gene promoter compared with the H/R group. These results confirmed that decreased HDAC2 binding to the miR-30a-5p promoter facilitated H3K14 hyperacetylation in senescent cardiomyocytes under HPostC treatment.

HDAC2-mediated H3K14 hyperacetylation inhibits senescent cardiomyocyte autophagy under HPostC. To determine whether HDAC2 was involved in the regulation of miR-30a-5p transcription in senescent cardiomyocytes, HDAC2 was knocked down in senescent cardiomyocytes. As presented in Fig. 2A and B, HDAC2 knockdown in senescent cardiomyocytes enhanced the enrichment of H3K14 acetylation levels at the miR-30a-5p gene promoter, which resulted in miR-30a-5p upregulation (Fig. 2C). Additionally, the expression of autophagy-related proteins LC3II/I, BECN1 and p62 were detected by western blotting to investigate the effects of HDAC2 on senescent cardiomyocyte autophagy. The results showed that HDAC2 knockdown significantly repressed LC3B-II/I and BECN1 expression, while increasing p62 expression under HPostC treatment (Fig. 2D). Subsequently, cell viability staining was performed, which exhibited that the viability of senescent cardiomyocytes exposed to H/R or HPostC significantly increased following romidepsin treatment, which suggested that H3K14 hyperacetylation facilitated the protection exerted by HPostC against H/R injury (Fig. 2E). These results demonstrated that HDAC2-mediated H3K14 hyperacetylation under HPostC treatment could facilitate miR-30a-5p expression to inhibit senescent cardiomyocyte autophagy.

c-Myc binds to the miR-30a-5p gene promoter to positively regulate miR-30a-5p transcriptional activity. To investigate whether c-Myc participated in the transcriptional regulation of miR-30a-5p, the possible c-Myc binding sites at the miR-30a-5p gene promoter were analyzed using the JASPAR database. Five putative transcription factor c-Myc binding sites was found at the miR-30a-5p gene core promoter (-761 to -216): i) -664/-673; ii) -326/-335; iii) -294/-303; iv) -263/-272; and v) -218/-227. Three binding sites with high scores were selected for subsequent experiments (Figs. 3A and S2). Subsequently, substitution mutations of the three identified c-Myc binding sites (-326/-335, -263/-272 and -218/-227) were generated and a luciferase reporter assay was performed to confirm which binding site was functionally required for c-Myc to regulate

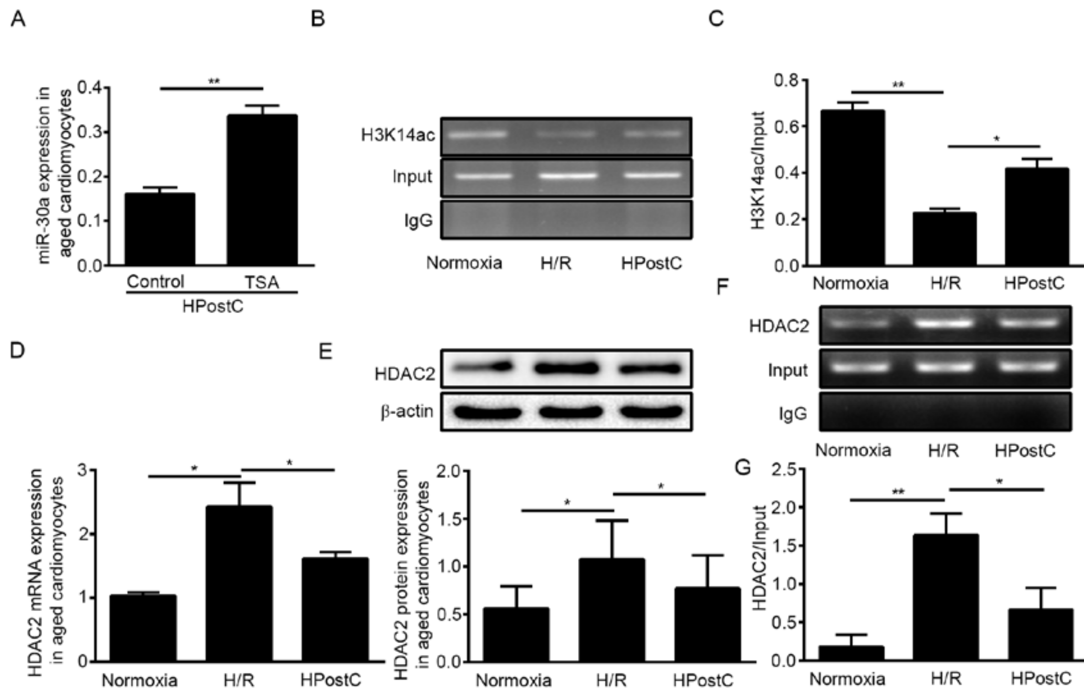

Figure 1. HPostC promotes H3K14 hyperacetylation at the miR-30a gene promoter via the downregulation of HDAC2 in senescent cardiomyocytes. (A) The mRNA expression of miR-30a-5p in senescent cardiomyocytes after treatment with TSA. (B and C) The enrichment of H3K14 acetylation at miR-30a-5p gene promoter was identified by ChIP-PCR analysis in the senescent cardiomyocytes. (D and E) The HDAC2 mRNA and protein expression levels in senescent cardiomyocytes. (F and G) HDAC2 binding at the miR-30a-5p gene promoter was analyzed by ChIP-PCR in senescent cardiomyocytes. Data are presented as the mean ± SD from three independent experiments. *P<0.05, **P<0.01. HPostC, hypoxia postconditioning; miR, microRNA; TSA, trichostatin A; HDAC2, histone deacetylase 2; ChIP, chromatin immunoprecipitation; H/R, hypoxia/reoxygenation.

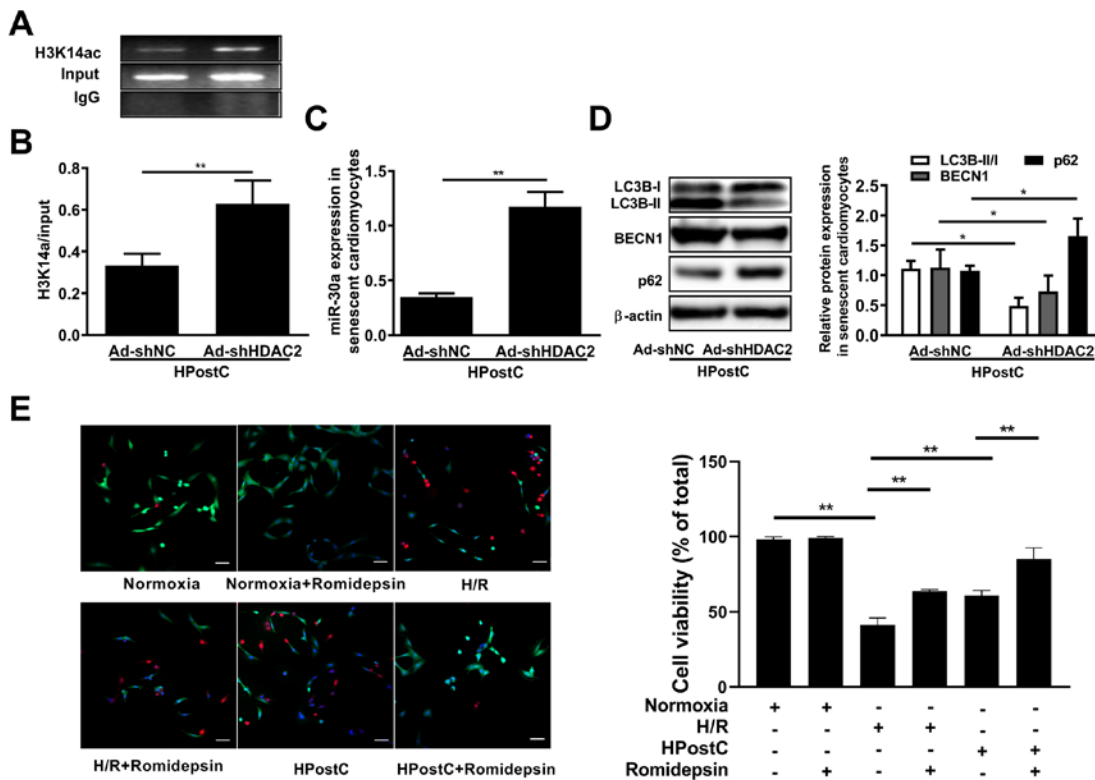

Figure 2. miR-30a-5p negatively regulated by HDAC2 is involved in HPostC-induced autophagy inhibition. (A and B) The enrichment of H3K14 acetylation at the miR-30a-5p gene promoter was identified by chromatin immunoprecipitation-PCR analysis in cells infected with Ad-shNC and Ad-shHDAC2 for 48 h. (C) miR-30a-5p mRNA expression in senescent cardiomyocytes treated as aforementioned. (D) The relative protein expression levels of LC3B-II/I, BECN1 and p62 after knockdown of HDAC2. (E) The cell viability staining of romidepsin-treated aged H9C2 cell under normoxia, H/R or HPostC conditions (scale bar, 50 μm). Data are presented as the mean ± SD from three independent experiments. *P<0.05, **P<0.01. HPostC, hypoxia postconditioning; miR, microRNA; HDAC2, histone deacetylase 2; H/R, hypoxia/reoxygenation; Ad-, adenovirus; sh, short hairpin RNA; NC, negative control; LC3B, light chain 3β; BECN1, beclin-1.

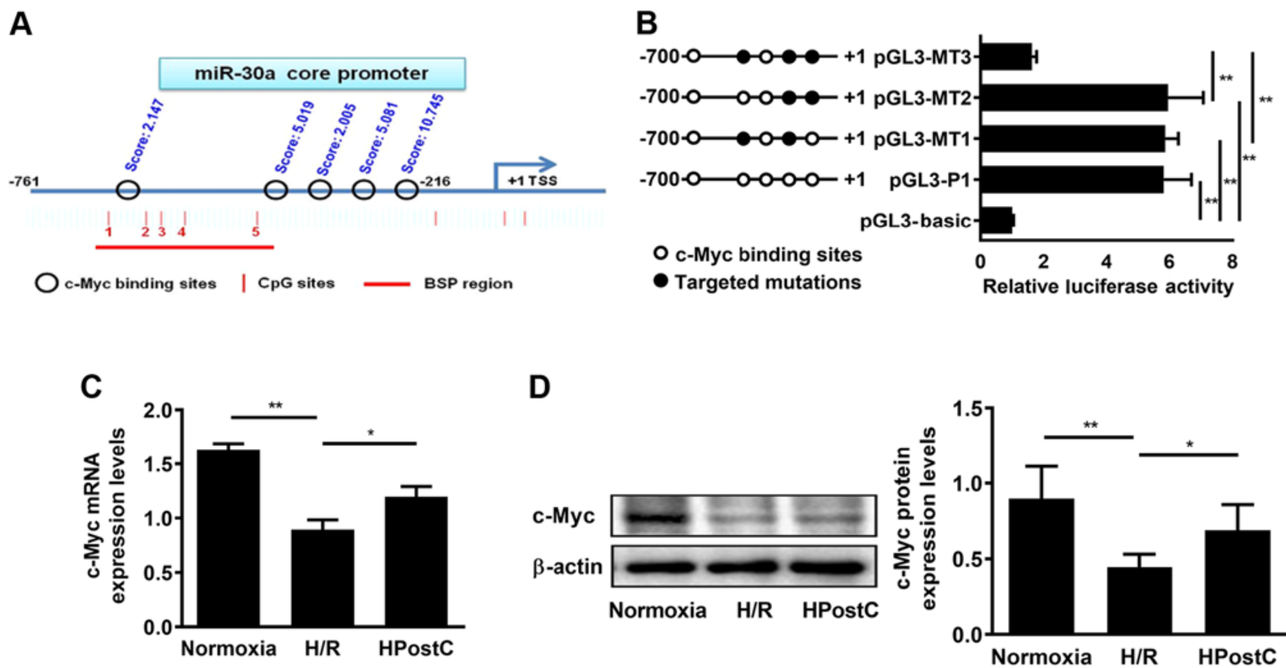

Figure 3. c-Myc positively regulates miR-30a-5p transcriptional activity. (A) Schematic diagram shows the locations of the predicted c-Myc binding sites (black hollow circle) surrounded by 5 CpG sites (red vertical bars) in the miR-30a-5p gene core promoter sites (-216/-761). (B) Sequential deletion and substitution mutation analyses identified c-Myc-responsive regions at the miR-30a-5p gene promoter region, miR-30a-5p transcriptional activities in 293T cells were detected using a luciferase reporter assay after solely or serially truncated c-Myc binding sites at the miR-30a-5p promoter region. (C and D) Reverse transcription-quantitative PCR and western blotting were performed to determine c-Myc expression in the senescent cardiomyocytes. Data are presented as the mean ± SD from three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$. HPostC, hypoxia postconditioning; miR, microRNA; H/R, hypoxia/reoxygenation.

miR-30a-5p transcriptional activity. pGL3-P1, which contained all c-Myc-binding sites, presented maximum promoter activity (Fig. 3B). Mutation of the region containing the -326/-335 and -263/-272 site (pGL3-MT1) and -263/-272 and -218/-227 (pGL3-MT2) showed similar promoter activity as pGL3-P1 (Fig. 3B). In addition, a significant reduction in miR-30a-5p gene promoter activity was observed when three identified binding sites were all mutated (pGL3-MT3) compared with pGL3-MT1 and pGL3-MT2 (Fig. 3B). These results demonstrated that both -218/-227 and -326/-335 regions were essential for c-Myc to regulate miR-30a-5p gene promoter activity. Meanwhile, c-Myc expression was measured using RT-qPCR and western blotting, and the results showed that HPostC significantly increased c-Myc expression compared with H/R (Fig. 3C and D). These results suggested that c-Myc may positively regulate miR-30a-5p gene promoter transcriptional activity in senescent cardiomyocytes under HPostC treatment.

H3K14 hyperacetylation facilitates c-Myc binding to the miR-30a-5p gene promoter to inhibit autophagy. To further verify the effect of c-Myc on the regulation of miR-30a-5p, senescent cardiomyocytes were infected with Ad-shRNA to knockdown c-Myc expression (Fig. S3). As expected, knockdown of c-Myc significantly inhibited the expression of miR-30a-5p (Fig. 4A), which was accompanied by increased LC3B-II/I and BECN1 expression and downregulation of p62 expression under HPostC treatment (Fig. 4B). This suggested that c-Myc may be involved in the inhibition of autophagy in senescent cardiomyocytes under HPostC treatment. In addition, cell viability staining showed that the viability of senescent cardiomyocytes were attenuated by c-Myc knockdown under H/R or HPostC conditions, which suggested that silencing of c-Myc promoted the protection exerted by HPostC against H/R injury (Fig. 4C). To determine the association between H3K14 hyperacetylation and c-Myc in miR-30a-5p transcription, senescent cardiomyocytes were treated with romidepsin, and c-Myc binding at the miR-30a-5p gene promoter, as well as miR-30a-5p expression, was examined by ChIP and RT-qPCR. As shown in Fig. 4D and E, romidepsin treatment significantly enhanced c-Myc binding to the miR-30a-5p gene promoter, which led to the increased expression of miR-30a-5p. Silencing c-Myc expression under romidepsin treatment suppressed c-Myc binding to the miR-30a-5p gene, and decreased the expression of miR-30a-5p. Collectively, these data suggested that H3K14 hyperacetylation promoted c-Myc binding to the miR-30a-5p gene promoter, which increased miR-30a-5p transcription in senescent cardiomyocytes.

Discussion

Ischemic heart disease has resulted in increased morbidity and mortality worldwide (24). Numerous preclinical reports have indicated that aging increases the vulnerability of the heart to I/R injury, and studies have aimed to find ways to reduce cardiac myocyte death following I/R (25). For example, it has been shown that I/R damage is likely to be the consequence of enhanced oxidative stress with ageing (26). Griecsová *et al* (27) demonstrated that the loss of preconditioning protection was associated with an age-dependent reduction of Akt phosphorylation and endothelial nitric oxide synthase and protein kinase C ε levels in the hearts of mature rats compared with the younger rats. Furthermore, it has been demonstrated that

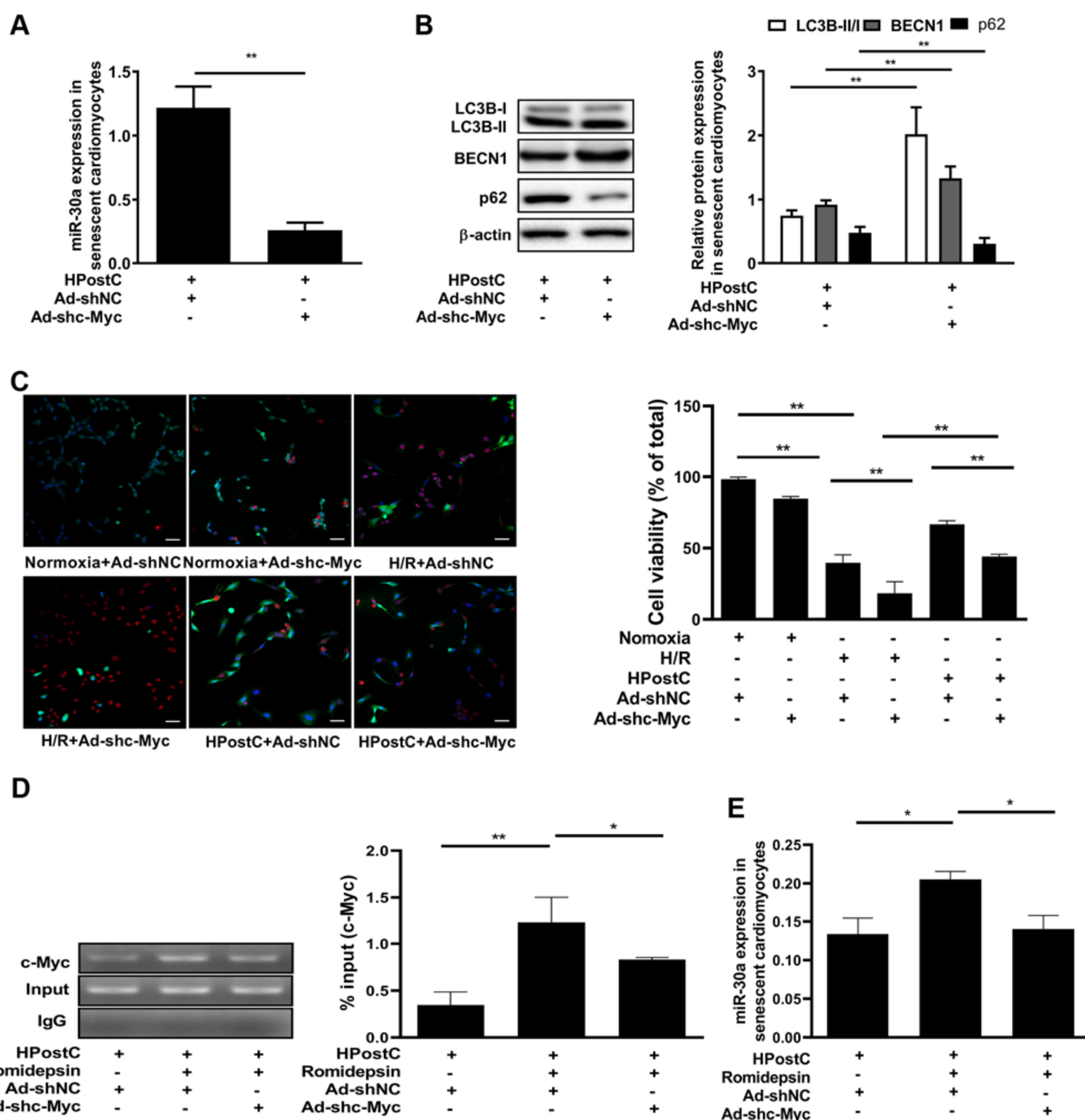

Figure 4. Acetylation of H3K14 promotes c-Myc binding to the miR-30a-5p gene promoter to inhibit autophagy of senescent cardiomyocytes. (A) miR-30a-5p mRNA expression was detected after knockdown of c-Myc expression under HPostC treatment. (B) Relative protein detection in the senescent cardiomyocytes treated as aforementioned. (C) Live/dead cell imaging in senescent cardiomyocytes treated as aforementioned (scale bar, 50 μm). (D) Chromatin immunoprecipitation-PCR assay of c-Myc binding at the miR-30a-5p gene promoter. (E) miR-30a-5p mRNA expression was examined by reverse transcription-quantitative PCR. Data are presented as the mean ± SD from three independent experiments. *P<0.05, **P<0.01. HPostC, hypoxia postconditioning; miR, microRNA; H/R, hypoxia/reoxygenation; Ad-, adenovirus; sh, short hairpin RNA; NC, negative control; LC3B, light chain 3β; BECN1, beclin-1.

aged myocytes accumulate more diastolic Ca^{2+} in ischemia and early reperfusion than younger hearts, cells may account for the increased sensitivity to ischemia and reperfusion injury in the aging heart (28). Myocardial IPostC is an endogenous cardioprotective phenomenon that can increase the tolerance of the heart to reperfusion injury when exposed to short-term I/R (29). Although the protection of IPostC on myocardial I/R injury has been confirmed, the underlying mechanisms in senescent myocardium are still unclear.

Autophagy is a vital physiological process in cells, which is considered the key to maintaining the normal structure and function of the heart (30). It has been reported that autophagy plays a dual role in myocardial I/R, low levels of autophagy can relieve energy depletion, maintain protein homeostasis, remove damaged cells and play a protective role in cell survival during ischemia (31), but long-term upregulation of autophagy can lead to excessive cell degradation and death (32). First, autophagy can effectively remove inflammasome and inhibit the activity of inflammatory transcription factors, such as NF-κB (33). However, excessive autophagy may lead to the release of inflammatory factors (34). On the other hand, using the autophagy inhibitor 3-MA or knocking down BECN1

expression can reduce cell death during myocardial I/R (35), and autophagy-related proteins Atg5 and BECN1 may turn into pro-apoptotic proteins in the case of being proteolyzed by proteases such as calpain (36). However, whether autophagy plays a beneficial or harmful role in myocardial I/R injury are still controversial. In the present study, autophagy was discovered to perform a destructive role in I/R injury.

Clinical studies have revealed significant prognostic benefits of IPostC in elderly patients with acute MI. For example, it has been reported that the peak of creatine kinase isoenzyme, post-operative myocardial troponin I and high sensitive C reaction protein are significantly attenuated by post-conditioning when compared with the control group (37). Similarly, it has also been found that 43 patients with ST-segment elevation myocardial infarction who underwent post-conditioning had a significant reduction in infarct size (38), which was similar to our previous study demonstrating that HPostC protected senescent H9C2 cells from H/R injury via DNMT3B-dependent miR-30a-5p activation (8). To further investigate the underlying mechanisms of miR-30a-5p upregulation in senescent cardiomyocytes under HPostC treatment, the present study explored another epigenetic modification that potentially involves miR-30a-5p transcriptional regulation. In this study, it was found that HDAC2-mediated H3K14 hyperacetylation contributed to miR-30a-5p upregulation in HPostC. In recent years, accumulating evidence has demonstrated that miR-30a-5p participates in cardiovascular pathophysiology (39-41). Although several studies have indicated the importance of miR-30a-5p in cardiovascular diseases, there are still conflicting views. Previous studies indicated that miR-30a may be a compensatory upregulation to protect the myocardium of patients with heart failure (42). Li *et al* (43) found that miR-30a was significantly decreased in I/R conditions, and knockdown of miR-30a could reverse the anti-autophagy effects of salvianolic acid B against I/R injury. By contrast, Shen *et al* (44) indicated that miR-30 was upregulated in a murine MI model and a cardiomyocyte hypoxic model. Another study also reported that the expression of circulating miR-30a in patients with MI was significantly elevated, which was also demonstrated to be a potential predictor of acute MI (45).

In addition to DNA methylation, the present study confirmed that the hyperacetylation of H3K14 at the miR-30a-5p gene promoter was responsible for the abnormal transcription of miR-30a-5p in senescent cardiomyocytes subjected to HPostC. Histone acetylation is associated with an 'open' chromatin conformation that promotes transcription (46). Acetylation weakens the electrostatic interaction between DNA and histones in nucleosome fibers, which reduces DNA affinity and allows chromatin to adopt a more relaxed structure to recruit basic transcription mechanisms (47). For example, the acetylated histone markers H3K4ac, H3K39ac and H3K14ac are correlated with transcriptional activation (48). In addition, histone acetylation has been reported to be catalyzed via two primary mechanisms (49): HATs destroy interactions between the DNA and histones, enabling transcription factors to enter the DNA (50); and HDACs can coagulate chromatin and inhibit transcription (51). In the present study, it was observed that decreased HDAC2 binding at the miR-30a-5p gene promoter resulted in H3K14 hyperacetylation in senescent cardiomyocytes under HPostC treatment. These data supported the hypothesis that histone hyperacetylation at the gene promoter contributed to transcriptional activation.

Several studies have suggested that histone acetylation facilitates chromatin opening and promotes the binding of transcription factors to DNA (52,53). Therefore, the present study further investigated whether hyperacetylation of H3K14 in the miR-30a-5p gene promoter affected the ability of transcription factors to bind to miR-30a-5p promoter regions. The results showed that five c-Myc putative binding sites were found, and only-218/-227 and -326/-335 sites were essential for c-Myc to regulate miR-30a-5p gene promoter activity at the miR-30a-5p gene promoter. c-Myc positively regulated miR-30a-5p gene promoter transcriptional activity in senescent cardiomyocytes under HPostC treatment. As a result, the present study indicated that hyperacetylation of H3K14 promoted c-Myc binding to the miR-30a-5p gene promoter, which led to the upregulation of miR-30a-5p transcription in senescent cardiomyocytes under HPostC treatment. Zhang *et al* (54) reported that high acetylation of the glial cell-derived neurotrophic factor (GDNF) promoter region promoted GDNF transcription via increasing early growth response protein 1 binding to the GDNF promoter, which was similar to the findings of the current study.

In conclusion, the present study found that HDAC2-mediated H3K14 hyperacetylation of the miR-30a-5p gene promoter facilitated c-Myc binding to the miR-30a-5p gene promoter, which contributed to the protective effects of HPostC against H/R injury on senescent cardiomyocytes. These findings provided novel insights into the mechanism underlying HPostC-mediated abnormal transcription of miR-30a-5p in senescent cardiomyocytes.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LBX and HPZ contributed to the conception of the study, performed statistical analysis and wrote the manuscript. LBX, HPZ, YHW, WG and LYG performed the experiments and acquired data. ANY and SCM provided conceptual advice and interpreted the data. YY and KW mainly helped with data collection and study design. YDJ designed and supervised the study. LBX and HPZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Curran J, Burkhoff D and Kloner RA: Beyond reperfusion: Acute ventricular unloading and cardioprotection during myocardial infarction. J Cardiovasc Transl Res 12: 95-106, 2019.
2. Akhmedov A, Montecucco F, Costantino S, Vdovenko D, Schaub Clerigué A, Gaul DS, Burger F, Roth A, Carbone F, Liberale L, *et al*: Cardiomyocyte-specific JunD overexpression increases infarct size following ischemia/reperfusion cardiac injury by downregulating Sirt3. Thromb Haemost 120: 168-180, 2020.
3. Zhang YM, Zhang ZY and Wang RX: Protective mechanisms of quercetin against myocardial ischemia reperfusion injury. Front Physiol 11: 956, 2020.
4. Zhao R, Xie E, Yang X and Gong B: Alliin alleviates myocardial ischemia-reperfusion injury by promoting autophagy. Biochem Biophys Res Commun 512: 236-243, 2019.
5. Zhang XY, Huang Z, Li QJ, Zhong GQ, Meng JJ, Wang DX and Tu RH: Ischemic postconditioning attenuates the inflammatory response in ischemia/reperfusion myocardium by upregulating miR-499 and inhibiting TLR2 activation. Mol Med Rep 22: 209-218, 2020.
6. Wei L, Liang H, Mo M, Liu Z, Ye R, Ye H, Ouyang W, Yu W, Zhao W and Zhang X: The effect of remote ischemic postconditioning on autonomic function in patients with acute ischemic stroke: A randomized controlled trail. Complement Ther Med 54: 102541, 2020.
7. Zhang C, Liao P, Liang R, Zheng X and Jian J: Epigallocatechin gallate prevents mitochondrial impairment and cell apoptosis by regulating miR-30a/p53 axis. Phytomedicine 61: 152845, 2019.
8. Wang Y, Hao Y, Zhang H, Xu L, Ding N, Wang R, Zhu G, Ma S, Yang A, Yang Y, *et al*: DNA Hypomethylation of miR-30a mediated the protection of hypoxia postconditioning against aged cardiomyocytes hypoxia/reoxygenation injury through inhibiting autophagy. Circ J 84: 616-625, 2020.
9. Kao SH, Cheng WC, Wang YT, Wu HT, Yeh HY, Chen YJ, Tsai MH and Wu KJ: Regulation of miRNA biogenesis and histone modification by K63-polyubiquitinated DDX17 controls cancer stem-like features. Cancer Res 79: 2549-2563, 2019.
10. Rymen B, Kawamura A, Lambolez A, Inagaki S, Takebayashi A, Iwase A, Sakamoto Y, Sako K, Favero DS, Ikeuchi M, *et al*: Histone acetylation orchestrates wound-induced transcriptional activation and cellular reprogramming in Arabidopsis. Commun Biol 2: 404, 2019.
11. Wei F, Tang D, Li Z, Kashif MH, Khan A, Lu H, Jia R and Chen P: Molecular cloning and subcellular localization of six HDACs and their roles in response to salt and drought stress in kenaf (Hibiscus cannabinus L.). Biol Res 52: 20, 2019.
12. Yuan H, Li H, Yu P, Fan Q, Zhang X, Huang W, Shen J, Cui Y and Zhou W: Involvement of HDAC6 in ischaemia and reperfusion-induced rat retinal injury. BMC Ophthalmol 18: 300, 2018.
13. Zhao TC, Cheng G, Zhang LX, Tseng YT and Padbury JF: Inhibition of histone deacetylases triggers pharmacologic preconditioning effects against myocardial ischemic injury. Cardiovasc Res 76: 473-481, 2007.
14. Wang Y, Liu H, Liu X, Zhang X, Wu J, Yuan L, Du X, Wang R, Ma Y, Chen X, *et al*: Histone acetylation plays an important role in MC-LR-induced apoptosis and cycle disorder in SD rat testicular cells. Chemosphere 241: 125073, 2020.
15. Parrot C, Kurbegovic A, Yao G, Couillard M, Côté O and Trudel M: c-Myc is a regulator of the PKD1 gene and PC1-induced pathogenesis. Hum Mol Genet 28: 751-763, 2019.
16. Karagiannis P, Takahashi K, Saito M, Yoshida Y, Okita K, Watanabe A, Inoue H, Yamashita JK, Todani M, Nakagawa M, *et al*: Induced pluripotent stem cells and their use in human models of disease and development. Physiol Rev 99: 79-114, 2019.
17. Saravia J, Zeng H, Dhungana Y, Bastardo Blanco D, Nguyen TM, Chapman NM, Wang Y, Kanneganti A, Liu S, Raynor JL, *et al*: Homeostasis and transitional activation of regulatory T cells require c-Myc. Sci Adv 6: eaaw6443, 2020.
18. Zhang L, Fu Y and Guo H: c-Myc-induced long non-coding RNA small nucleolar RNA host gene 7 regulates glycolysis in breast cancer. J Breast Cancer 22: 533-547, 2019.
19. Lee EJ, Seo E, Kim JW, Nam SA, Lee JY, Jun J, Oh S, Park M, Jho EH, Yoo KH, *et al*: TAZ/Wnt-β-catenin/c-MYC axis regulates cystogenesis in polycystic kidney disease. Proc Natl Acad Sci USA 117: 29001-29012, 2020.
20. Chen S, Bu D, Ma Y, Zhu J, Chen G, Sun L, Zuo S, Li T, Pan Y, Wang X, *et al*: H19 overexpression induces resistance to 1,25(OH)2D3 by targeting VDR through miR-675-5p in colon cancer cells. Neoplasia 19: 226-236. 2017.
21. Guo W, Zhang H, Yang A, Ma P, Sun L, Deng M, Mao C, Xiong J, Sun J, Wang N, *et al*: Homocysteine accelerates atherosclerosis by inhibiting scavenger receptor class B member1 via DNMT3b/SP1 pathway. J Mol Cell Cardiol 138: 34-48, 2020.
22. UniProt C: UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 49: D480-D489, 2021.
23. Wen Z, Lian L, Ding H, Hu Y, Xiao Z, Xiong K and Yang Q: LncRNA ANCR promotes hepatocellular carcinoma metastasis through upregulating HNRNPA1 expression. RNA Biol 17: 381-394, 2020.
24. Chen K, Miller EJ and Sadeghi MM: PET-based imaging of ischemic heart disease. PET Clin 14: 211-221, 2019.
25. Li C, Mu N, Gu C, Liu M, Yang Z, Yin Y, Chen M, Wang Y, Han Y, Yu L and Ma H: Metformin mediates cardioprotection against aging-induced ischemic necroptosis. Aging Cell 19: e13096, 2020.
26. Webster I, Salie R, Marais E, Fan WJ, Maarman G, Huisamen B and Lochner A: Myocardial susceptibility to ischaemia/reperfusion in obesity: A re-evaluation of the effects of age. BMC Physiol 17: 3, 2017.
27. Griecsová L, Farkašová V, Gáblovský I, Khandelwal VK, Bernátová I, Tatarková Z, Kaplan P and Ravingerová T: Effect of maturation on the resistance of rat hearts against ischemia. Study of potential molecular mechanisms. Physiol Res 64 (Suppl): S685-S696, 2015.
28. O'Brien JD, Ferguson JH and Howlett SE: Effects of ischemia and reperfusion on isolated ventricular myocytes from young adult and aged Fischer 344 rat hearts. Am J Physiol Heart Circ Physiol 294: H2174-H1783, 2008.
29. Bayrami G, Karimi P, Agha-Hosseini F, Feyzizadeh S and Badalzadeh R: Effect of ischemic postconditioning on myocardial function and infarct size following reperfusion injury in diabetic rats pretreated with vildagliptin. J Cardiovasc Pharmacol Ther 23: 174-183, 2018.
30. Chávez MN, Morales RA, López-Crisosto C, Roa JC, Allende ML and Lavandero S: Autophagy activation in zebrafish heart regeneration. Sci Rep 10: 2191: 2020.
31. Shi B, Ma M, Zheng Y, Pan Y and Lin X: mTOR and Beclin1: Two key autophagy-related molecules and their roles in myocardial ischemia/reperfusion injury. J Cell Physiol 234: 12562-12568, 2019.
32. Torres-Esquivel C, Montiel T, Flores-Méndez M and Massieu L: Effect of β-hydroxybutyrate on autophagy dynamics during severe hypoglycemia and the hypoglycemic coma. Front Cell Neurosci 14: 547215, 2020.
33. Chen C, Li J, Zhang W, Shah SWA and Ishfaq M: Mycoplasma gallisepticum triggers immune damage in the chicken thymus by activating the TLR-2/MyD88/NF-κB signaling pathway and NLRP3 inflammasome. Vet Res 51: 52, 2020.
34. Huang Y, Liao Y, Zhang H and Li S: Lead exposure induces cell autophagy via blocking the Akt/mTOR signaling in rat astrocytes. J Toxicol Sci 45: 559-567, 2020.
35. Liu Y, Song A, Wu H, Sun Y and Dai M: Paeonol inhibits apoptosis of vascular smooth muscle cells via up-regulation of autophagy by activating class III PI3K/Beclin-1 signaling pathway. Life Sci 264: 118714, 2021.
36. Campbell GR, Bruckman RS, Chu YL, Trout RN and Spector SA: SMAC mimetics induce autophagy-dependent apoptosis of HIV-1-infected resting memory CD4+ T cells. Cell Host Microbe 24: 689-702.e7, 2018.

37. Zhang J, Zhang X, Cui Y, Ferdous M, Cui L and Zhao P: Different postconditioning cycles affect prognosis of aged patients undergoing primary percutaneous coronary intervention. Cardiol J 25: 666-673, 2018.
38. Slettom G, Jonassen AK, Dahle GO, Seifert R, Larsen TH, Berge RK and Nordrehaug JE: Insulin postconditioning reduces infarct size in the porcine heart in a dose-dependent manner. J Cardiovasc Pharmacol Ther 22: 179-188, 2017.
39. Maciejak A, Kostarska-Srokosz E, Gierlak W, Dluzniewski M, Kuch M, Marchel M, Opolski G, Kiliszek M, Matlak K, Dobrzycki S, *et al*: Circulating miR-30a-5p as a prognostic biomarker of left ventricular dysfunction after acute myocardial infarction. Sci Rep 8: 9883, 2018.
40. Lv XB, Niu QH, Zhang M, Feng L and Feng J: Critical functions of microRNA-30a-5p-E2F3 in cardiomyocyte apoptosis induced by hypoxia/reoxygenation. Kaohsiung J Med Sci 37: 92-100, 2021.
41. Ding H, Wang Y, Hu L, Xue S, Wang Y, Zhang L, Zhang Y, Qi H, Yu H, Aung LHH, *et al*: Combined detection of miR-21-5p, miR-30a-3p, miR-30a-5p, miR-155-5p, miR-216a and miR-217 for screening of early heart failure diseases. Biosci Rep 40: BSR20191653, 2020.
42. Zhao DS, Chen Y, Jiang H, Lu JP, Zhang G, Geng J, Zhang Q, Shen JH, Zhou X, Zhu W and Shan QJ: Serum miR-210 and miR-30a expressions tend to revert to fetal levels in Chinese adult patients with chronic heart failure. Cardiovasc Pathol 22: 444-450, 2013.
43. Li D, Wang J, Hou J, Fu J, Liu J and Lin R: Salvianolic acid B induced upregulation of miR-30a protects cardiac myocytes from ischemia/reperfusion injury. BMC Complement Altern Med 16: 336, 2016.
44. Shen Y, Shen Z, Miao L, Xin X, Lin S, Zhu Y, Guo W and Zhu YZ: miRNA-30 family inhibition protects against cardiac ischemic injury by regulating cystathionine-γ-lyase expression. Antioxid Redox Signal 22: 224-240, 2015.
45. Long G, Wang F, Duan Q, Yang S, Chen F, Gong W, Yang X, Wang Y, Chen C and Wang DW: Circulating miR-30a, miR-195 and let-7b associated with acute myocardial infarction. PLoS One 7: e50926, 2012.
46. Kubiak M, Jurek A, Kamińska K, Kowalewski J, Huang S and Lewandowska MA: Chromosome conformation capture reveals two elements that interact with the PTBP3 (ROD1) transcription start site. Int J Mol Sci 20: 242, 2019.
47. Lee J and Lee TH: How protein binding sensitizes the nucleosome to histone H3K56 acetylation. ACS Chem Biol 14: 506-515, 2019.
48. Hu Y, Lai Y, Chen X, Zhou DX and Zhao Y: Distribution pattern of histone marks potentially determines their roles in transcription and RNA processing in rice. J Plant Physiol 249: 153167, 2020.
49. Narita T, Weinert BT and Choudhary C: Functions and mechanisms of non-histone protein acetylation. Nature reviews. Mol Cell Biol 20: 156-174, 2019.
50. Okonkwo A, Mitra J, Johnson GS, Li L, Dashwood WM, Hegde ML, Yue C, Dashwood RH and Rajendran P: Heterocyclic analogs of sulforaphane trigger DNA damage and impede DNA repair in colon cancer cells: Interplay of HATs and HDACs. Mol Nutr Food Res 62: e1800228, 2018.
51. Han KA, Shin WH, Jung S, Seol W, Seo H, Ko C and Chung KC: Leucine-rich repeat kinase 2 exacerbates neuronal cytotoxicity through phosphorylation of histone deacetylase 3 and histone deacetylation. Hum Mol Genet 26: 1-18, 2017.
52. Bascom GD and Schlick T: Chromatin fiber folding directed by cooperative histone tail acetylation and linker histone binding. Biophys J 114: 2376-2385, 2018.
53. Church M, Smith KC, Alhussain MM, Pennings S and Fleming AB: Sas3 and Ada2(Gcn5)-dependent histone H3 acetylation is required for transcription elongation at the de-repressed FLO1 gene. Nucleic Acids Res 45: 4413-4430, 2017.
54. Zhang BL, Guo TW, Gao LL, Ji GQ, Gu XH, Shao YQ, Yao RQ and Gao DS: Egr-1 and RNA POL II facilitate glioma cell GDNF transcription induced by histone hyperacetylation in promoter II. Oncotarget 8: 45105-45116, 2017.

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