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ORIGINAL RESEARCH

Dioscin alleviates cardiomyocyte pyroptosis in acute myocardial infarction rats via regulating LncRNA *FGD5-AS1*/*miR-424*/HOXA3 axis

Songjie Bi[1,†], Wei Chen[1,†], Qi Wu[1,*], Hong Yang[1], Meng Jia[1], Chunmei Liu[1]

[1]Department of Cardiology, The Second Affiliated Hospital of Chengdu Medical College, Nuclear Industry 416 Hospital, 610051 Chengdu, Sichuan, China

***Correspondence**
wuqi8371157@126.com
(Qi Wu)

[†] These authors contributed equally.

Abstract

Acute myocardial infarction (AMI) is a severe cardiovascular condition. Recently, it has been discovered that dioscin plays pivotal roles in the domains of anti-inflammatory, antiviral, and anti-tumor activities. However, Nevertheless, the precise impact and mechanism by which dioscin protects against cardiomyocyte pyroptosis in AMI remains unclear. This study aimed to determine the significance and investigate the mechanisms by which dioscin affects cardiomyocyte pyroptosis in cases of acute myocardial infarction. In this study, rats were divided into four distinct groups: sham, AMI, AMI + Negative Control (NC), and AMI + FGD5 Antisense RNA 1 (*FGD5-AS1*). Subsequently, quantitative real-time polymerase chain reaction (qPCR) assay, quantification of infarct size, and enzyme linked immunosorbent assay (ELISA) assay were used to confirm the expression of *FGD5-AS1* after AMI surgeries. The results indicated that, expression of *FGD5-AS1* was significantly decreased. Furthermore, it was determined that *FGD5-AS1* can alleviate myocardial damage induced by AMI. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay and western blot assay results indicated that *FGD5-AS1* promoted cell proliferation and decreased the inflammatory response and pyroptosis of cardiomyocytes induced by hypoxia/re-oxygenation (H/R). In addition, a combination of bioinformatics approaches, dual-luciferase reporter assay, and RNA pull down assay were used to predict and confirm the interaction between *FGD5-AS1*, microRNA-424 (*miR-424*), and Homeobox A3 (HOXA3). It was observed that *FGD5-AS1* facilitates cell proliferation and alleviate H/R-induced inflammatory response and cardiomyocyte pyroptosis by regulating HOXA3 expression. Finally, the confirmation of dioscin's ability to mitigate myocardial damage and cardiomyocyte pyroptosis resulting from acute myocardial infarction (AMI) and regulating the expression of *FGD5-AS1*/*miR-424*/HOXA3 axis was established. Together, this research demonstrates that dioscin effectively reduces cardiomyocyte pyroptosis in rats with acute myocardial infarction (AMI) by modulating long non-coding RNA (LncRNA) *FGD5-AS1*/*miR-424*/HOXA3 axis.

Keywords

Acute myocardial infarction; Cardiomyocyte pyroptosis; Dioscin; LncRNA *FGD5-AS1*; *miR-424*; HOXA3

1. Introduction

Acute myocardial infarction (AMI) remains the most serious cardiac event, although mortality have steadily declined over the past few years [1, 2]. Inflammatory response and cardiomyocyte pyroptosis are the main features of acute myocardial infarction, and play a vital role in myocardial dysfunction and heart failure [3, 4]. Thus, understanding the molecular process of cell death (apoptosis) and the body's immune response (inflammatory response) in myocardial cells can provide new insights and treatment options for AMI therapy [5, 6].

Dioscin is a natural steroidal saponin that is commonly found in plants belonging to the diosgenaceae family [7]. Multiple studies have demonstrated that dioscin possesses anti-tumor properties [8]. Furthermore, research has indicated that dioscin can induce DNA damage [9], activate the mitochondrial signaling pathway and cause cell apoptosis, and effectively combat lung cancer [10]. Nevertheless, there is currently no available information on the impact of dioscin on acute myocardial infarction, and the precise molecular mechanism and pharmacodynamics underlying the anti-pyroptosis activity of dioscin on cardiomyocytes remains unexplored.

LncRNAs refer to endogenous cellular RNAs that are not

involved in coding for proteins. Although lncRNAs cannot be used as templates for protein synthesis, they have been shown to play a crucial role in numerous cardiovascular disorders [11, 12]. LncRNA cardiac hypertrophy-related factor (LncRNA CHRF) has been reported to be involved in cardiomyocyte function and promote cardiac hypertrophy through miR-93 regulation of AKT serine/threonine kinase 3 (*AKT3*) [13]. Downregulation of antisense noncoding RNA in the INK4 locus (*ANRIL*) can reduce cardiomyocyte apoptosis in AMI by regulating interleukin-33 (*IL-33*)/suppression of tumorigenicity 2 (*ST2*) [14]. Pro-cardiac fibrotic lncRNA (*PCFL*) promotes myocardial fibrosis after myocardial infarction through miR-378/growth factor receptor bound protein 2 (*GRB2*) pathway [15]. Furthermore, Shen *et al*. [16] suggested that the expression of *FGD5-AS1* was reduced in individuals with acute myocardial infarction. This finding indicates that *FGD5-AS1* could serve as a novel regulatory marker for acute myocardial infarction, as revealed through the study of important mRNAs and lncRNAs using integrated network analysis [16].

FGD5-AS1, also known as FGD5 antisense RNA 1, a novel long-stranded non-coding RNA, has been reported to be decreased in the oxygen glucose deprivation/re-oxygenation (OGD/R) model of nerve cells, and overexpression can facilitate the survival of nerve cells, reduce apoptosis and play a neuroprotective role [17]. *FGD5-AS1* expression is also reduced in periodontitis and lipopolysaccharides (LPS)-induced periodontal ligament cells (PDLCS) cells, and upregulation can reduce LPS-induced inflammation by miR-142-3p/suppressor of cytokine signaling 6 (*SOCS6*)/nuclear factor kappa-B (NF-κB) signaling [18]. However, the role and mechanism of *FGD5-AS1* in myocardial infarction injury is still unknown. StarBase predicted that *FGD5-AS1* could bind to *miR-424* and *miR-424* can regulate cardiomyocyte pyrolysis and promote myocardial ischemia/reperfusion injury. In addition, StarBase predicted that *miR-424* could target HOXA3, and HOXA3 indirectly regulated myocardial apoptosis through transcriptional inhibition of NOD-like receptor thermal protein domain associated protein 3 (NLRP3) expression. This study mainly explored the effect of lncRNA *FGD5-AS1* on myocardial infarction through *miR-424*/HOXA3 by dioscin.

2. Materials and methods

2.1 Animals

8 weeks old male Sprague-Dawley rats (245–275 g) were provided by SIPPR-Bk Laboratory Animals Co. Ltd. (Shanghai, China). All the protocols used were ratified by the Animal Ethics Committee of the Second Affiliated Hospital of Chengdu Medical College in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [19]. Each rat was fed 5 g/100 g of fresh dry feed and about 10 mL/100 g of acidified water with a pH of 2.5–2.8 per day. The duration of light exposure was 12 hours per day, and the rats were prevented from being stimulated by bright light (Approval No. 20230137).

2.2 AMI rat administration

Twenty-four Sprague–Dawley rats were divided randomly into four groups: sham, AMI, AMI + NC, and AMI + *FGD5-AS1*, (n = 6). Rats in the AMI groups underwent surgery to cause AMI [20]. On the first day post AMI treated by surgical ligation of the left anterior descending coronary artery, rats in AMI + NC and AMI + *FGD5-AS1* groups were treated intravenously. Briefly, LncRNA *FGD5-AS1* and NC (GenePharma, Shanghai, China) were intravenously injected into the tail vein of the rats. On the first day after AMI treated by surgical ligation of the left anterior descending coronary artery, rats in AMI and AMI + dioscin groups were treated intravenously. Briefly, dioscin (100 mg/kg) and equal volume of 0.9% saline were intravenously injected into the tail vein of rats, respectively.

2.3 qPCR

The expression of LncRNA *FGD5-AS1*, *miR-424*, and HOXA3 were detected using SYBR Premix EX Taq (RR390Q, Takara, Kusatsu, Japan). The relative expression of LncRNA *FGD5-AS1*, *miR-424*, and HOXA3 were analyzed by the $2^{-\Delta\Delta Ct}$ method. Primer sequences are shown in Table 1.

TABLE 1. Primers for LncRNA *FGD5-AS1*, *miR-424*, HOXA3, and reference genes.

Gene	Primer	Sequence (5′ → 3′)
FGD5 Antisense RNA 1		
	Forward	AGAAGCGGAGGGGTGAAAAT
	Reverse	CCGCCTTATAGTTGGCCCTC
microRNA-424		
	Forward	GCAGCAGCAATTCATGTTT
	Reverse	GTGCAGGGTCCGAGGT
Homeobox A3		
	Forward	CACGCGGAGCGAAACAGT
	Reverse	CAGTCCTCCGTTTGCTGG
β-actin		
	Forward	GTGACGTTGACATCCGTAAAGA
	Reverse	GCCGGACTCATCGTACTCC

2.4 Quantification of infarct size (2,3,5,Triphenyl-2H-Tetrazolium Chloride staining)

The obtained myocardial tissues were cut into 3 mm slices and incubated with 2% TTC solution. Then, the slices were fixed with 8% formalin and photographed. The infarct size was quantified by ImageJ (1.53a, National Institutes of Health, Montgomery Village, MD, USA).

2.5 ELISA

For detecting the lactate dehydrogenase (LDH), IL-1β and IL-18 protein levels, ELISA kits (LDH kit, ab102526, Abcam, Waltham, MA, USA; IL-1β kit, ab100767, Abcam, Waltham,

MA, USA; IL-18 kit, ab213909, Abcam, Waltham, MA, USA) were used. The production of LDH, IL-1β and IL-18 in the serum of rats were detected following the manufacturer's instructions.

2.6 Cell culture

Rat cardiomyocyte H9C2 were purchased from the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified eagle medium (DMEM) (11965092, Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS), and 1% p/s in incubator.

2.7 Cell administration

Rat cardiomyocyte H9C2 were seeded in serum-free medium and exposed to hypoxia condition in incubator with 5% carbon dioxide and 95% nitrogen or normoxia condition in 5% carbon dioxide and 95% oxygen. On the first day post H/R treatment, Rat cardiomyocyte H9C2 in H/R and H/R + dioscin groups were treated by adding supplements to the culture medium. Briefly, dioscin (2 μg/mL) and equal volume of DMSO were added into the culture medium, respectively.

2.8 Cell transfection

The synthetic LncRNA *FGD5-AS1*, *miR-424* mimics, NC mimics (control), *miR-424* inhibitor, NC inhibitor (control) and HOXA3-targeting short hairpin RNA (shRNA) oligonucleotide sequences were acquired from GenePharma (Shanghai, China). LncRNA *FGD5-AS1* and NC were transfected into cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

2.9 MTT assay

Cells in 96-well plates were incubated for 4 h with 0.6 mg/mL MTT solution (C0009S, Beyotime, Shanghai, China). Subsequently, the culture medium was replaced by 100 μL dimethyl sulfoxide (DMSO, D8418, Sigma-Aldrich, St. Louis, MO, USA) to visualize. The optical density was detected at 490 nm by microplate reader (Epoch 2, BioTek, Winooski, VT, USA).

2.10 Western blot

Briefly, radio immunoprecipitation assay (RIPA) lysis buffer (P0013B, Beyotime, Shanghai, China) was applied to extract the total protein from cells (Beyotime, Shanghai, China). Bicinchoninic acid assay (BCA) kit (CW00145, CoWin Biotechnology, Jiangsu, China) was used to determine the concentration of protein, which was then electrophoresed to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, protein was transferred to the polyvinylidene difluoride (PVDF) membranes followed by non-fat milk. The membranes were incubated with specific primary antibodies NOD-like receptor thermal protein domain associated protein 3 (NLRP3, ab214185, 1:2500, Abcam, Waltham, MA, USA), apoptosis associated speck like protein containing a CARD (ASC, ab180799, 1:2500, Abcam, Waltham, MA, USA), cleaved caspase-1 (ab62698, 1:3000, Abcam, Waltham, MA, USA), gasdermin (GSDMD-N, ab219800, 1:3000, Abcam, Waltham, MA, USA), HOXA3 (ab230879, 1:3000, Abcam, Waltham, MA, USA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, ab9485, 1:2000, Abcam, Waltham, MA, USA), and β-actin (ab8227, 1:2500, Abcam, Waltham, MA, USA) overnight at 4 °C. Afterwards, the membranes were further incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (ab205718, 1:3000, Abcam, Waltham, MA, USA). The samples were normalized by β-actin and analysed using ImageJ software.

2.11 Dual-luciferase reporter assay

The fragments of 3′-UTRs of *FGD5-AS1* or HOXA3 mRNA binding sites of *miR-424* were cloned into pmirGLO luciferase report vector (E1330, Promega, Madison, WI, USA). *MiR-424* mimics and NC mimics were co-transfected with reporter plasmids into cells by Lipofectamine 2000. After two days of transfection, the luciferase activity was detected by the dual-luciferase kit (E1910, Promega, Madison, WI, USA).

2.12 RNA pull-down assay

FGD5-AS1 sense and NC (control) probes coupled with biotin were synthesized by GenePharma (Shanghai, China). Cells were treated in RNA immunoprecipitation lysis buffer with streptavidin coated magnetic beads. Subsequently, the lysates were incubated with probe-coated magnetic beads and washed using wash buffer. qPCR was applied to check *miR-424* expression in the RNA complex bound to the beads.

2.13 Statistical analysis

All data are demonstrated as the mean ± standard error of the mean from 6 independent repeats. Comparisons among different groups with one independent variable were performed using one-way analysis of variance (ANOVA) and comparisons among different groups with two independent variables were performed using two-way ANOVA.

3. Results

3.1 *FGD5-AS1* alleviates myocardial damage caused by acute myocardial infarction

The expression of LncRNA *FGD5-AS1* was assessed in order to examine the correlation between *FGD5-AS1* and AMI. The findings demonstrated a significant decrease in the expression of *FGD5-AS1* following AMI surgeries. Nevertheless, the rats that received intravenous injection of *FGD5-AS1* exhibited a noticeable increase in the expression of *FGD5-AS1*, suggesting that the intravenous injection effectively up-regulated the expression level of *FGD5-AS1* (Fig. 1A). Next, the impact of *FGD5-AS1* on acute myocardial infarction (AMI) was evaluated by measuring the size of infarcted tissue in rats. The results demonstrated a clear increase in infarction volume due to AMI therapy. Nevertheless, the excessive expression of *FGD5-AS1* significantly decreased the volume of infarction, as depicted in (Fig. 1B). In addition, ELISA was employed to assess the protein expression of lactate dehydrogenase (LDH) in rats, in order to investigate the impact of *FGD5-AS1* on the

mitigation of cardiac damage. The ELISA results indicated a significant rise in the protein expression level of LDH in the AMI group. *FGD5-AS1* clearly reduced the protein expression of LDH, as seen in (Fig. 1C). These results proved that *FGD5-AS1* mitigates myocardial damage caused by acute myocardial infarction.

3.2 *FGD5-AS1* reduces H/R-induced cardiomyocyte pyroptosis

In this study, cardiomyocytes H9C2 were selected to study the regulatory role of *FGD5-AS1* on AMI. Compared with cells cultured under normal condition, cells cultured under H/R condition had lower expression level of *FGD5-AS1*. *FGD5-AS1* or NC were transfected into H9C2 and cultured under H/R conditions. The expression of *FGD5-AS1* in the cells transfected with *FGD5-AS1* was significantly elevated, suggesting that the transfection successfully up-regulated the expression of *FGD5-AS1* (Fig. 2A). Subsequently, the MTT test was utilized to evaluate the impact of *FGD5-AS1* on the cellular proliferation of H9C2. The findings suggested that H/R treatment significantly suppressed cell proliferation, whereas *FGD5-AS1* greatly enhanced the cell proliferation capacity of H9C2 (Fig. 2B).

Subsequently, ELISA was utilized to assess the protein expression of inflammation-related factors (IL-18 and IL-1β) in H9C2 cells that overexpressed *FGD5-AS1*, in order to evaluate the inflammatory response. The results indicated that the protein expression of IL-18 and IL-1β was significantly increased in the H/R group, whereas it was significantly decreased in the *FGD5-AS1* group. This suggests that *FGD5-AS1* can minimize the inflammatory response (Fig. 2C). Furthermore, western blot analysis was conducted to examine inflammation and cell pyroptosis-related proteins. The findings indicated that the H/R treatment significantly increased the expression of NLRP3, ASC, cleaved caspase-1, and GSDMD-N. Conversely, the overexpression of *FGD5-AS1* notably reduced the levels of these inflammation and cell pyroptosis-related proteins (Fig. 2D). These results suggested *FGD5-AS1* reduces H/R-induced cardiomyocyte pyroptosis.

3.3 *FGD5-AS1* targets *miR-424* expression

To explore the biomechanism of *FGD5-AS1* in AMI, the mRNA binding sites were anticipated in StarBase (https://rnasysu.com/encori/). The results suggested that lncRNA *FGD5-AS1* was a target of *miR-424*. The forecasted 3′-UTRs of *FGD5-AS1* binding to *miR-424* is presented in (Fig. 3A). The findings validated that the increased expression of *miR-424* significantly decreased the luciferase activity of the *FGD5-AS1*-wt reporter gene, as shown in (Fig. 3B). To further attest whether *FGD5-AS1* regulated *miR-424* expression, RNA pulldown assay was conducted. The results proved that *miR-424* was significantly enriched in *FGD5-AS1* group than NC group (Fig. 3C). Additionally, a qPCR test was utilized to elucidate the correlation between *FGD5-AS1* and *miR-424*. Cells cultured under H/R condition had higher expression levels of *miR-424*. However, the expression of *miR-424* in the cells transfected with *FGD5-AS1* was significantly decreased, suggesting that *FGD5-AS1* has the ability to regulate the expression of *miR-424* (Fig. 3D).

3.4 *FGD5-AS1* regulates HOXA3 expression by targeting *miR-424*

The mRNA binding sites were forecasted in StarBase once more to find out the binding target of *miR-424*. The results indicated that HOXA3 was a target of *miR-424*. The forecasted 3′-UTRs of HOXA3 mRNA binding to *miR-424* is presented in (Fig. 4A). The results confirmed the overexpression of *miR-424* markedly reduced the luciferase activity of the HOXA3-wt reporter gene (Fig. 4B). The findings indicated that the introduction of *miR-424* mimics significantly suppressed the expression of HOXA3 mRNA and protein. Conversely, the use of a *miR-424* inhibitor noticeably increased the production of HOXA3 mRNA and protein (Fig. 4C,D). To further prove

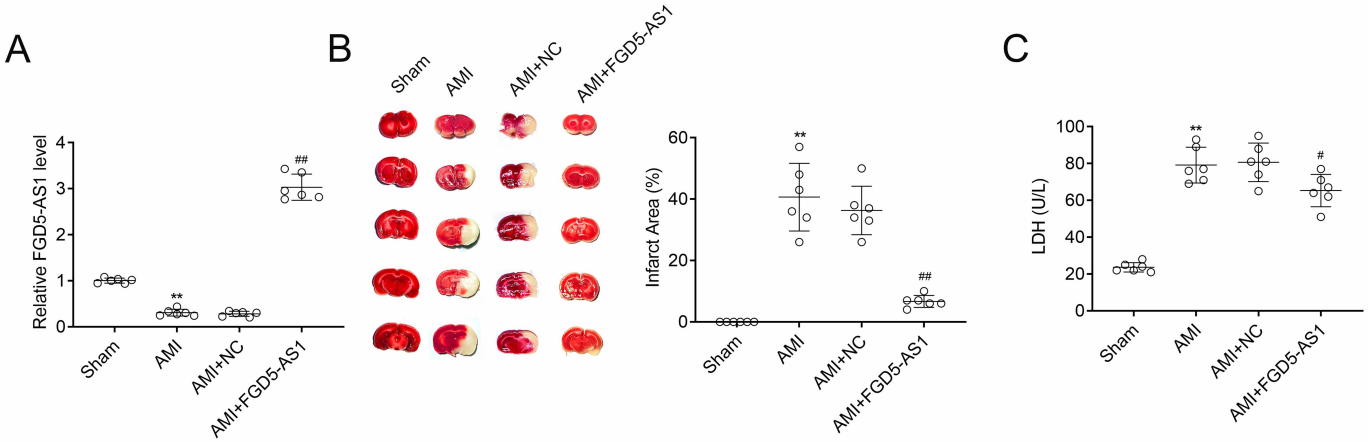

FIGURE 1. *FGD5-AS1* alleviates myocardial damage caused by acute myocardial infarction. (A) The mRNA expression levels of *FGD5-AS1* in rats in each group. (B) Infarction volume was assessed to check protective effect of *FGD5-AS1* on AMI rats in each group. (C) The protein expression levels of LDH of rats in each group. Data were presented as the mean ± SD with three independent experiments. $^{**}p < 0.01$ versus sham group, $^{\#}p < 0.05$, and $^{\#\#}p < 0.01$ versus AMI + NC group. AMI: Acute myocardial infarction; LDH: lactate dehydrogenase; *FGD5-AS1*: FGD5 Antisense RNA 1; NC: Negative control.

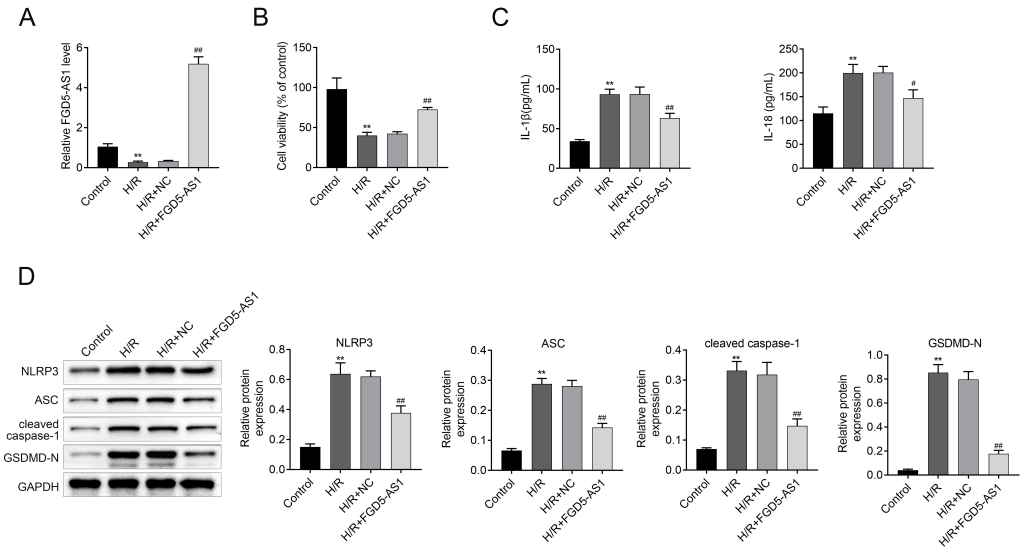

FIGURE 2. *FGD5-AS1* reduces H/R-induced cardiomyocyte pyroptosis. (A) The mRNA expression levels of *FGD5-AS1* of H9C2 in each group. (B) Cell proliferation ability of H9C2 was detected using MTT assay. (C) The protein expression levels of IL-18 and IL-1β of H9C2 in each group. (D) Western blot was applied to detect inflammation and cell pyroptosis-related proteins in H9C2 in each group. Data were presented as the mean ± SD with three independent experiments. $^{**}p < 0.01$ versus control group, $^{\#}p < 0.05$, and $^{\#\#}p < 0.01$ versus H/R + NC group. *FGD5-AS1*: FGD5 Antisense RNA 1; MTT: methylthiazolyldiphenyl-tetrazolium bromide; IL-18: interleukin-18; IL-1β: interleukin-1β; H/R: hypoxia/re-oxygenation; NC: Negative control; NLRP3: NOD-like receptor thermal protein domain associated protein 3; ASC: apoptosis associated speck like protein containing a CARD; GSDMD-N: gasdermin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

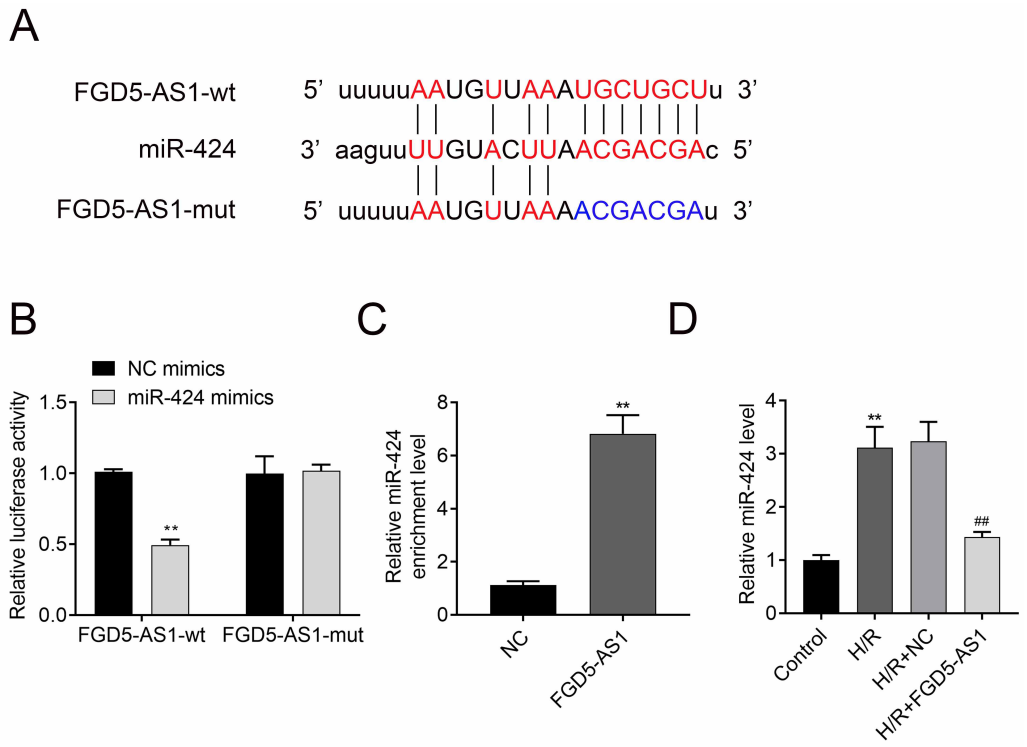

FIGURE 3. *FGD5-AS1* targets *miR-424* expression. (A) Forecast of *miR-424* binding sites on target gene *FGD5-AS1* by StarBase. (B) Dual-luciferase assays were carried out after cells were co-transfected *FGD5-AS1*-wt or *FGD5-AS1*-mut with *miR-424* mimics and NC mimics (control), respectively. (C) RNA pulldown experiment showed that *miR-424* was significantly enriched for *FGD5-AS1*. (D) The mRNA expression levels of *miR-424* of H9C2 in each group. Data were presented as the mean ± SD with three independent experiments. $^{**}p < 0.01$ versus control group and $^{\#\#}p < 0.01$ versus H/R + NC group. *FGD5-AS1*: FGD5 Antisense RNA 1; NC: Negative control; H/R: hypoxia/re-oxygenation.

whether *FGD5-AS1* regulates HOXA3 expression by targeting *miR-424*, the expression of HOXA3 protein was checked in the three types of cells, including H9C2 transfected NC + NC mimics, *FGD5-AS1*+ NC mimics, and *FGD5-AS1*+ *miR-424* mimics. Western blot results indicated that *FGD5-AS1* significantly enhanced the expression of HOXA3 protein, while *miR-424* mimics strongly inhibited the expression of BCL2L13 protein (Fig. 4E). These data suggested that *FGD5-AS1* regulates HOXA3 expression by specifically targeting *miR-424*.

3.5 *FGD5-AS1* alleviates H/R-induced cardiomyocyte pyroptosis by regulating HOXA3 expression

MTT assay was utilized to detect cell proliferation of H9C2. Prior MTT assays have demonstrated that H/R treatment dramatically suppressed cell proliferation, whereas *FGD5-AS1* greatly enhanced the cell proliferation of H9C2. Nevertheless, the cell proliferation of H9C2 with suppressed HOXA3 expression significantly decreased. Remarkably, the growth and division of H9C2 cells with suppressed HOXA3 gene expression significantly increased following the introduction of *FGD5-AS1* by transfection (Fig. 5A). Subsequently, the expression level of inflammation-related factors (IL-18 and IL-1β) were assessed by ELISA to check the influence of *FGD5-AS1* on H9C2 through HOXA3. Previous ELISA assay has showed that the protein expression of IL-18 and IL-1β were significantly increased in the H/R group, while the protein expression of IL-18 and IL-1β were significantly decreased in the *FGD5-AS1* group.

Nevertheless, the protein expression levels of IL-18 and IL-1β were significantly elevated in H9C2 cells with reduced HOXA3. Notably, the protein expression of IL-18 and IL-1β in HOXA3 knocked down H9C2 cells was significantly reduced following transfection with *FGD5-AS1*, as seen in (Fig. 5B). Furthermore, western blot analysis was conducted to examine inflammation and cell pyroptosis-related proteins. The findings demonstrated a significant increase in the expression of NLRP3, ASC, cleaved caspase-1, and GSDMD-N following H/R treatment. Conversely, the overexpression of *FGD5-AS1* resulted in a substantial downregulation of these inflammation and cell pyroptosis-related proteins. Nevertheless, the levels of inflammation and cell pyroptosis-related proteins in HOXA3 knocked down H9C2 cells were significantly elevated. Notably, the levels of inflammation and cell pyroptosis-related proteins in HOXA3 knocked down H9C2 cells were significantly reduced following transfection with *FGD5-AS1* (Fig. 5C). The data provided conclusive evidence that *FGD5-AS1* mitigates cardiomyocyte pyroptosis produced by H/R by modulating the expression of HOXA3.

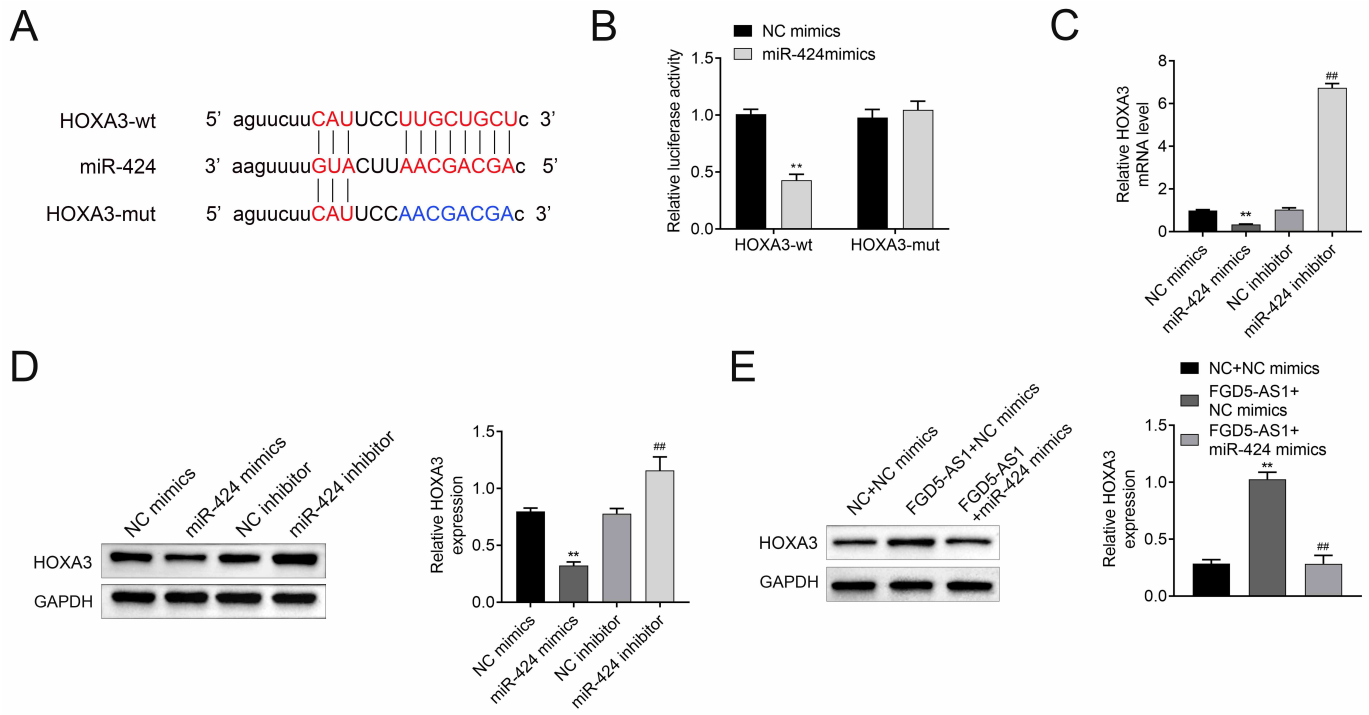

FIGURE 4. ***FGD5-AS1* regulates HOXA3 expression by targeting *miR-424*.** (A) Forecast of *miR-424* binding sites on target gene HOXA3 by StarBase. (B) Dual-luciferase assays were carried out after cells were co-transfected HOXA3-wt or HOXA3-mut with *miR-424* mimics and NC mimics (control), respectively. (C,D) The mRNA and protein expression levels of HOXA3 in H9C2 in each group. (E) The protein expression levels of HOXA3 in H9C2 in each group. Data were presented as the mean ± SD with three independent experiments. $^{**}p < 0.01$ versus control group and $^{\#\#}p < 0.01$ versus H/R + NC group. HOXA3: Homeobox A3; NC: Negative control; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; *FGD5-AS1*: FGD5 Antisense RNA 1.

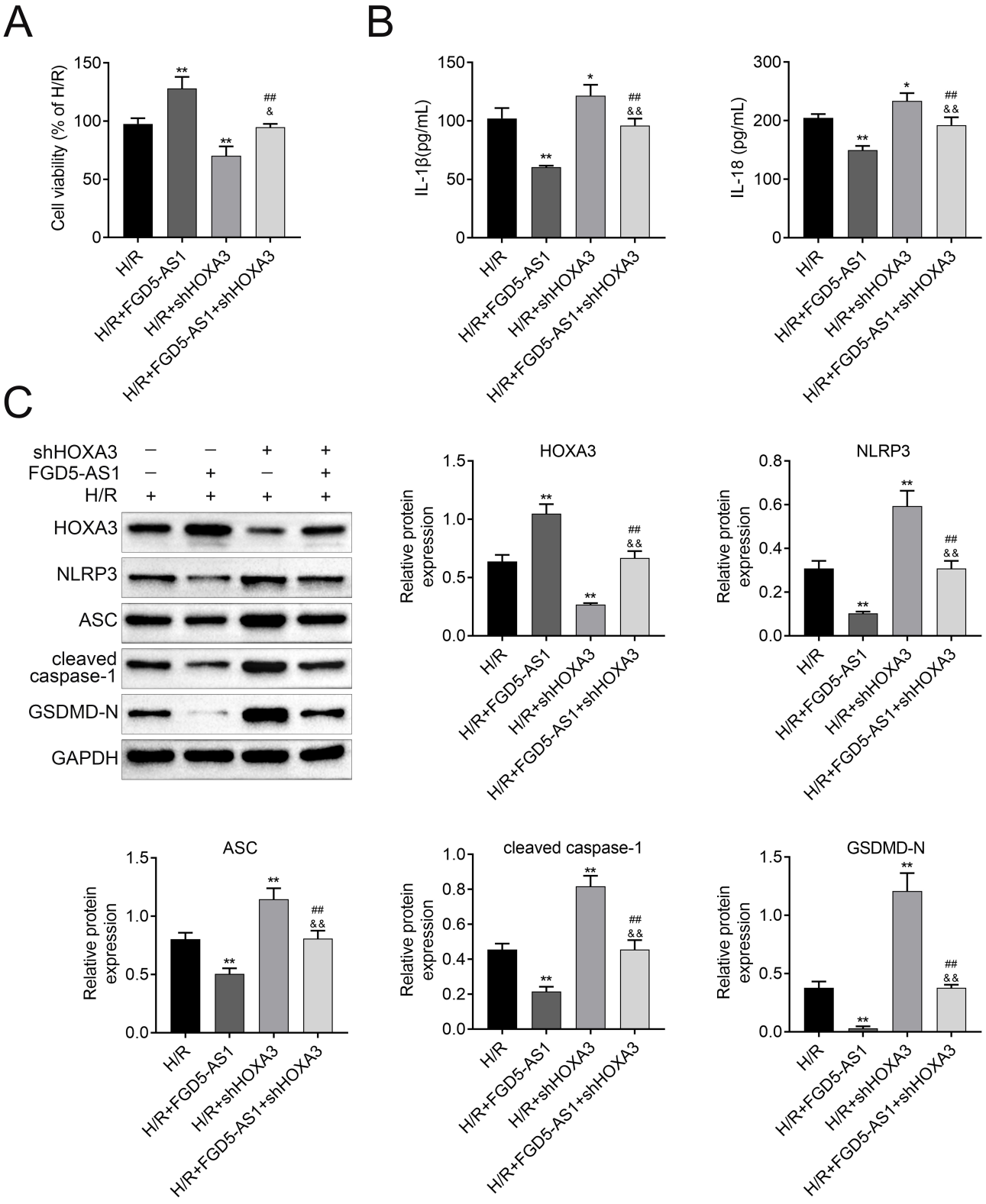

FIGURE 5. ***FGD5-AS1*** **alleviates H/R-induced cardiomyocyte pyroptosis by regulating HOXA3 expression.** (A) Cell proliferation ability of H9C2 was detected using MTT assay. (B) The protein expression of IL-18 and IL-1β of H9C2 in each group. (C) Western blot was applied to detect inflammation and cell pyroptosis-related proteins in H9C2 in each group. Data were presented as the mean ± SD with three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus H/R group; $^{\#\#}p < 0.01$ versus H/R + FGD5-AS1 group; $^{\&}p < 0.05$ and $^{\&\&}p < 0.01$ versus H/R + shHOXA3 group. *FGD5-AS1*: FGD5 Antisense RNA 1; H/R: hypoxia/re-oxygenation; HOXA3: Homeobox A3; MTT: methylthiazolyldiphenyl-tetrazolium bromide; IL-18: interleukin-18; IL-1β: interleukin-1β; NC: Negative control; NLRP3: NOD-like receptor thermal protein domain associated protein 3; ASC: apoptosis associated speck like protein containing a CARD; GSDMD-N: gasdermin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

3.6 Dioscin alleviates myocardial damage and cardiomyocyte pyroptosis caused by acute myocardial infarction

The impact of dioscin on acute myocardial infarction (AMI) was evaluated by measuring the volume of infarction in rats. The results demonstrated a clear enhancement in infarction volume due to AMI therapy. Nevertheless, dioscin significantly decreased the volume of infarction (Fig. 6A). In addition, ELISA was employed to assess the protein expression of LDH in rats in order to examine the impact of dioscin on the mitigation of myocardial damage. The ELISA results indicated a significant rise in the protein expression level of LDH in the AMI group. However, it is evident that dioscin significantly reduced the protein expression of LDH, as seen in (Fig. 6B). Subsequently, the MTT assay was utilized to assess the impact of dioscin on the cellular proliferation of H9C2. The findings suggested that the treatment with H/R significantly suppressed cell proliferation, but dioscin greatly enhanced the cell proliferation ability of H9C2 (Fig. 6C).

Subsequently, the ELISA findings suggested a significant increase in the protein expression of IL-18 and IL-1β in the H/R group. Conversely, the protein expression of IL-18 and IL-1β showed a notable decrease in the dioscin group, showing that dioscin has the ability to attenuate inflammatory response (Fig. 6D). In addition, inflammation and cell pyroptosis-related proteins were also analysed by western blot, and the results showed that H/R treatment markedly upregulated the expression of NLRP3, ASC, cleaved caspase-1, and GSDMD-N, while overexpression of dioscin significantly downregulated these inflammation and cell pyroptosis-related proteins (Fig. 6E,F). These results suggested that dioscin alleviates myocardial damage and reduces cardiomyocyte pyroptosis by AMI.

3.7 Dioscin regulates the expression of *FGD5-AS1*/*miR-424*/HOXA3 axis

To further attest whether dioscin regulated *FGD5-AS1*/*miR-424*/HOXA3 expression, qPCR assay was conducted in both AMI rats and H9C2 cells. The findings demonstrated a notable drop in the expression level of *FGD5-AS1* and HOXA3, while *miR-424* increased significantly in the AMI or H/R group. However, the expression level of *FGD5-AS1*, *miR-424*, and HOXA3 was reversed after treatment with dioscin, suggesting that dioscin regulates the expression of *FGD5-AS1*/*miR-424*/HOXA3 axis (Fig. 7A,B).

4. Discussion

Despite numerous remarkable advancements in modern medicine, the molecular pathways responsible for acute myocardial infarction (AMI) remain poorly comprehended. Recent investigations have indicated that dioscin has the potential to play a significant role in reducing inflammation and preventing cell death (apoptosis) [21]. Nevertheless, there is limited research on the effects of dioscin in AMI. Investigating the molecular regulation mechanism of dioscin in AMI could potentially provide new treatment targets for this condition. Furthermore, the precise molecular regulatory mechanism by which dioscin influences the determination of AMI destiny is still not well comprehended. Recent investigations have suggested that lncRNAs may have the capacity to play a crucial role in cell proliferation and intracellular trafficking [22]. However, there are few studies on lincRNAs in AMI, and understanding the mechanism of LncRNA *FGD5-AS1* in AMI could potentially provide a novel therapeutic target for the treatment of AMI.

Dioscin has garnered significant interest in the medical domain in recent years. Traditionally, professionals frequently utilize it as a treatment for wound healing, hemostasis, inflammation reduction, and cancer prevention. Research has demonstrated that dioscin exhibits significant anti-tumor properties [23]. Several studies have demonstrated that dioscin has the ability to specifically target the activation of miR-149-3p expression, inhibit the AKT1/p53 signaling pathway, cause apoptosis of pancreatic cancer cells, and effectively combat pancreatic cancer [24]. Other studies have shown that dioscin can activate estrogen receptor estrogen receptor β and induce apoptosis of prostate cancer cells (PC3) and prostate stem cells [25]. Here, the results showed that dioscin alleviates myocardial damage and reduces cardiomyocyte pyroptosis by AMI. Further, we explored the specific biological mechanism of dioscin in alleviating pyroptosis in AMI rats.

LncRNA *FGD5-AS1* is an endogenous cellular RNA that been confirmed to play a pathological role in many diseases. *FGD5-AS1* has been found to impact the advancement of cardiovascular disorders and other ailments and found to promote the spread and growth of renal cell carcinoma through the extracellular regulated protein kinases (ERK)/AKT signaling pathway [26]. A recent study shown that the interaction between *FGD5-AS1* and microRNA (miRNA)-223 reduces neuronal damage [17]. Furthermore, it has been revealed that *FGD5-AS1* can mitigate the advancement of glioblastoma through the activation of the Wnt/β-catenin signaling pathway [27]. A review on the association between LncRNA *FGD5-AS1* and cancer has shown that *FGD5-AS1* is closely related to lymph node metastasis, tumor invasion, survival time, and recurrence rate of various cancers. Mechanism analysis showed that *FGD5-AS1* induced cancer cell proliferation, metastasis, invasion and drug resistance *in vitro*, and promoted tumor growth and metastasis *in vivo* through stable mRNA expression of spongifying miRNA.

In addition, *FGD5-AS1* can be used as a diagnostic or prognostic marker for a variety of cancers [28]. The expression abundance of most lncRNAs is low, which greatly limits their functional scope and influence. However, *FGD5-AS1* is a highly expressed lncRNA [29]. *FGD5-AS1*, a promising lncRNA, has a wide range of targets and hence holds great potential for the creation of new and safe therapeutic techniques. However, limited study has focused on the profitability of *FGD5-AS1* in AMI, and the probable mechanism has not been elucidated. This study shown that *FGD5-AS1* can improve myocardium damage caused by acute myocardial infarction (AMI). Furthermore, *FGD5-AS1* has the ability to reduce cardiomyocyte pyroptosis produced by H/R. These findings indicate that *FGD5-AS1* may have a role in enhancing symptoms of AMI.

Mounting evidence has emphasized that lncRNA can me-

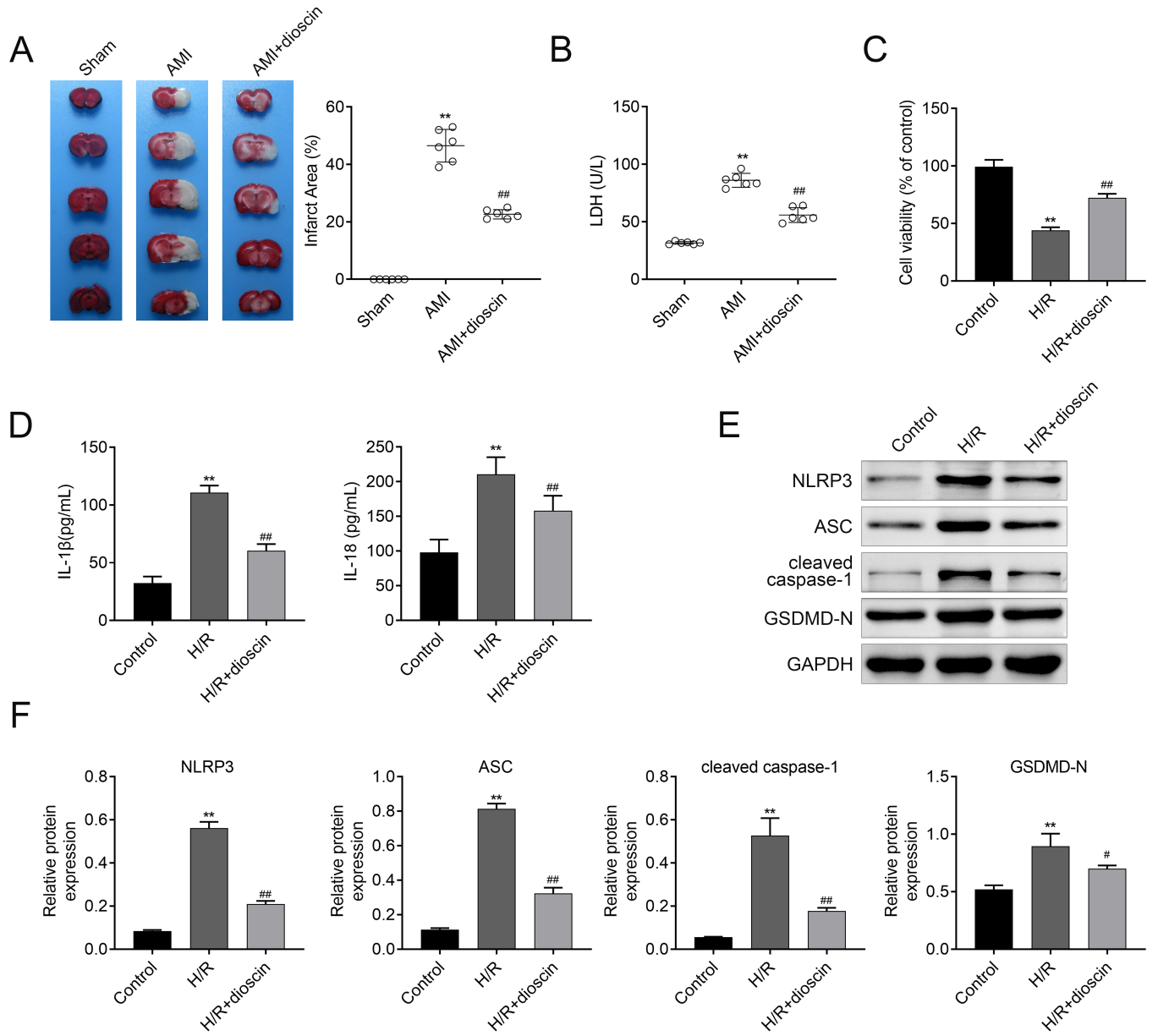

FIGURE 6. Dioscin alleviates myocardial damage and cardiomyocyte pyroptosis caused by acute myocardial infarction. (A) Infarction volume was assessed to check protective effect of dioscin on AMI rats in each group. (B) The protein expression levels of LDH of rats in each group. (C) Cell proliferation ability of H9C2 was detected using MTT assay. (D) The protein expression levels of IL-18 and IL-1β of H9C2 in each group. (E) Western blot was applied to detect inflammation and cell pyroptosis-related proteins in H9C2 in each group. (F) Statistics of relative protein expression levels of inflammation and cell pyroptosis-related proteins in H9C2 in each group. Data were presented as the mean ± SD with three independent experiments. $^{**}p < 0.01$ versus sham group, $^{\#}p < 0.05$, and $^{\#\#}p < 0.01$ versus AMI + dioscin group. AMI: Acute myocardial infarction; LDH: lactate dehydrogenase; H/R: hypoxia/re-oxygenation; IL: interleukin; NLRP3: NOD-like receptor thermal protein domain associated protein 3; ASC: apoptosis associated speck like protein containing a CARD; GSDMD-N: gasdermin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

diate the expression of target microRNAs to achieve specific physiological functions. A prior work shown that *FGD5-AS1* promotes cisplatin resistance in lung cancer cells through miR-142-5p [30]. Moreover, FGD5-AS1 was showed to increase the invasion and migration of glioblastoma cells via miR-103a-3p [31]. Furthermore, it has been demonstrated that *FGD5-AS1* influences the progression of periodontitis through its interaction with miR-142-3p [18]. Another new finding of this study is that *FGD5-AS1* targets *miR-424* expression.

Many researches showed that microRNA achieve their functions by mediating the expression of target mRNAs [32]. *MiR-424* has been reported to regulate cardiomyocyte pyroptosis via targeting of CRISPLD2 [33]. In addition, *miR-424* has been demonstrated to regulate cell cycle and inhibit cell proliferation via targeting E2F7 [34]. The confirmation of *miR-424*'s ability to target HOXA3 was achieved using luciferase activity assay, western blot assays, and qPCR assay. Moreover, it has been verified that *FGD5-AS1* enhances the production of HOXA3

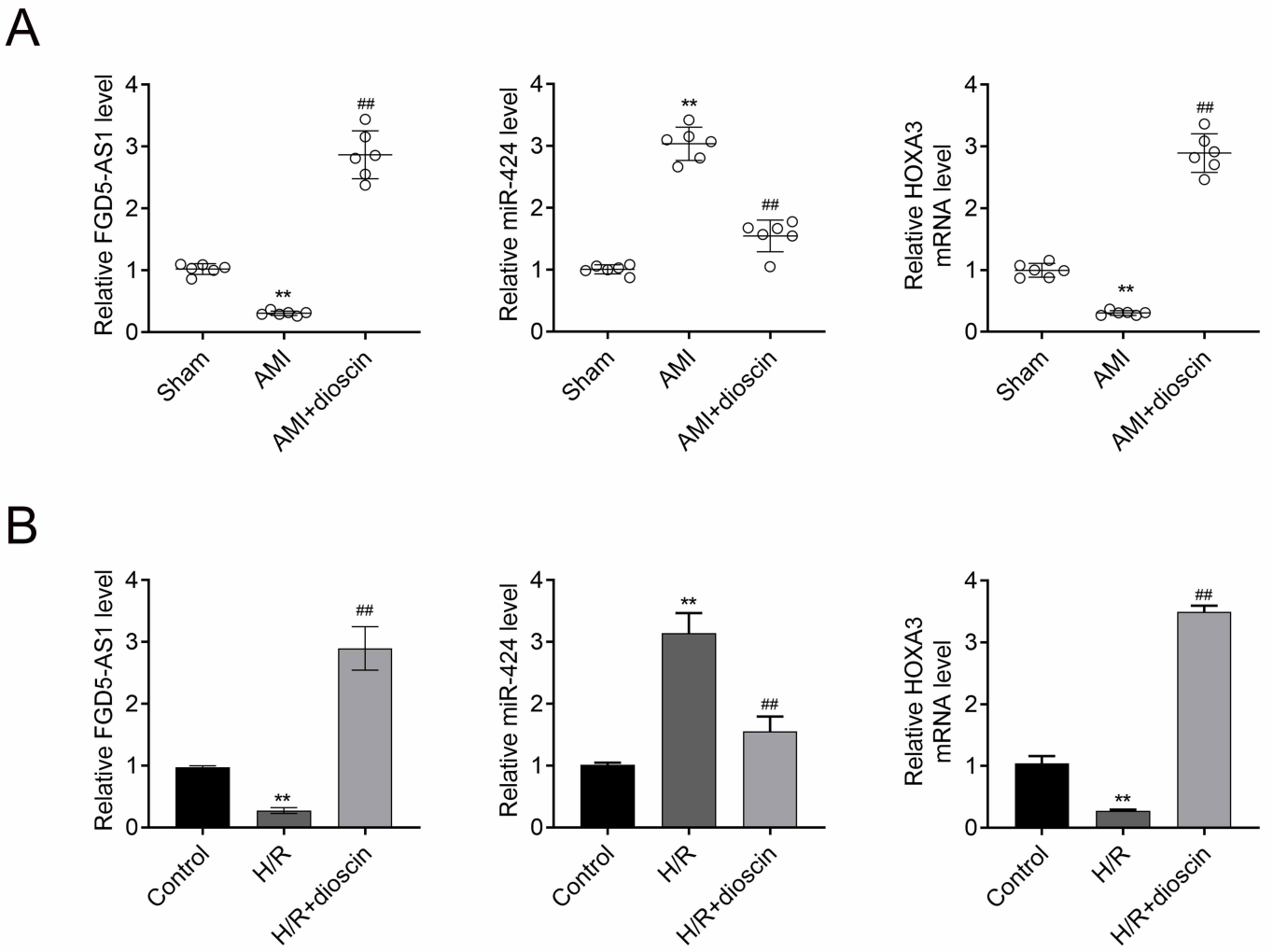

FIGURE 7. Dioscin regulates the expression of *FGD5-AS1*/*miR-424*/HOXA3 axis. (A,B) The mRNA expression levels of *FGD5-AS1*, *miR-424*, and HOXA3 of AMI rats and H9C2 in each group. Data were presented as the mean ± SD with three independent experiments. $^{**}p < 0.01$ versus control group and $^{\#\#}p < 0.01$ versus dioscin group. AMI: Acute myocardial infarction; *FGD5-AS1*: FGD5 Antisense RNA 1; HOXA3: Homeobox A3; H/R: hypoxia/re-oxygenation.

protein, whereas *miR-424* suppresses the production of B-cell lymphoma-2 Like 13 (BCL2L13) protein. This confirms that *FGD5-AS1* controls the expression of HOXA3 via targeting *miR-424*. Ultimately, it was verified that *FGD5-AS1* promotes cell growth and reduces the inflammatory response and pyroptosis in cardiomyocytes produced by hypoxia/reoxygenation (H/R) via regulating the expression of *miR-424*/HOXA3.

5. Conclusions

In conclusion, we discovered that *FGD5-AS1* can ameliorate myocardial damage caused by AMI. Moreover, *FGD5-AS1* could significantly decrease H/R-induced cardiomyocyte pyroptosis, regulates HOXA3 expression by targeting *miR-424*, and alleviates H/R-induced cardiomyocyte pyroptosis by regulating HOXA3 expression. Finally, it has been demonstrated that dioscin effectively alleviates myocardial damage and cardiomyocyte pyroptosis caused by AMI, and regulates the expression of *FGD5-AS1*/*miR-424*/HOXA3 axis. The findings indicate that dioscin effectively reduces cardiomyocyte pyroptosis in rats with acute myocardial infarction (AMI) by modulating the LncRNA *FGD5-AS1*/*miR-424*/HOXA3 axis. Future studies should look at *FGD5-AS1*, *miR-424*, and HOXA3 as possible biomarkers of AMI and cardiomyocyte pyroptosis and evaluate their application value in early diagnosis and efficacy monitoring. In addition, clinical trials can be conducted to verify the efficacy and safety of dioscin in patients with AMI and explore its feasibility in clinical applications. Ultimately, novel drugs can be developed based on the *FGD5-AS1*/*miR-424*/HOXA3 axis, whiles exploring its potential in the treatment of AMI, and optimizing its drug delivery system to improve therapeutic efficacy.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTIONS

SJB and WC—designed the study, supervised the data collection. QW—analyzed the data, interpreted the data. HY,

MJ and CML—prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of the Second Affiliated Hospital of Chengdu Medical College (Approval No. 20230137).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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