Acute pulmonary embolism (APE) is characterized by the occlusion of pulmonary arteries. Pulmonary embolism (PE) may present with typical features such as dyspnea and pleural chest pain, but it may also be less typical, for example, latent dyspnea or fainting for several days to weeks, with relatively few respiratory symptoms (1). For most patients with APE, direct oral anticoagulant therapy is effective and convenient in reducing the risk of bleeding, and for those with hemodynamic impairment, systemic thrombolytic therapy is a conservative treatment (2). Cardiac output is reduced as the right ventricle tries to overcome the increased afterload in APE (3), and sudden increased pulmonary vascular resistance causes acute right ventricular systolic dysfunction and further impairs transpulmonary blood flow (4). Therefore, the management of myocardial injury following APE is an essential part of APE treatment.

Curcumin is a substance extracted from the roots of the turmeric plant and is a lipophilic polyphenol that acts as an anti-inflammatory, antioxidant, and anti-apoptotic agent. It has been shown to have beneficial effects in various cardiovascular diseases. However, the direct effect of curcumin on APE has rarely been studied. Therefore, the present study aimed to investigate the therapeutic potential of curcumin in APE and associated myocardial injury to provide new insights into curcumin as a promising competitive new target for the treatment of APE.

The treatment of patients with acute pulmonary embolism (APE) is extremely challenging due to the complex clinical presentation and prognosis of APE related to the patient’s hemodynamic status and insufficient arterial blood flow and right ventricular overload. Protective efficacy against cardiovascular diseases of curcumin, a common natural polyphenolic compound, which has antithrombotic properties and reduces platelet accumulation in the circulation by inhibiting thromboxane synthesis has been demonstrated. However, the direct effect of curcumin on APE has rarely been studied. Therefore, the present study aimed to investigate the therapeutic potential of curcumin in APE and associated myocardial injury to provide new insights into curcumin as a promising competitive new target for the treatment of APE.

A suspension of 12 mg/kg microspheres was injected intravenously into rats. An APE rat model was built. Before modeling, intragastric 100 mg/kg curcumin was given, and/or lentiviral plasmid vector targeting microRNA-145-5p or insulin receptor substrate 1 (IRS1) was injected. Pulmonary artery pressure was measured to assess right ventricular systolic pressure (RVSP). Hematoxylin and eosin (H&E) staining was performed on liver tissues and myocardial tissues of APE rats. TUNEL (terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling) staining and immunohistochemical (IHC) staining were conducted to measure apoptosis and CyPA-CD147 expression in the myocardium, respectively. Inflammatory indices interleukin-1beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) were measured by ELISA in cardiac tissues. RT-qPCR and Western blot were performed to determine the expression levels of related genes. In addition, by dual luciferase reporter assay and RIP assay, the relationship between microRNA-145-5p and insulin receptor substrate 1 (IRS1) was confirmed. In results: curcumin improved APE-induced myocardial injury, reduced myocardial tissue edema, and thrombus volume. It attenuated APE-induced myocardial inflammation and apoptosis, as well as reduced lung injury and pulmonary artery pressure. Curcumin promoted microRNA-145-5p expression in APE rat myocardium. MicroRNA-145-5p overexpression protected against APE-induced myocardial injury, and microRNA-145-5p silencing abolished the beneficial effects of curcumin in APE-induced myocardial injury. IRS1 was targeted by microRNA-145-5p. IRS1 silencing attenuated APE-induced myocardial injury, and enhanced therapeutic effect of curcumin on myocardial injury in APE rats. In conclusion, curcumin alleviates myocardial inflammation, apoptosis, and oxidative stress induced by APE by regulating microRNA-145-5p/IRS1 axis.
antibiotic, anti-inflammatory, and anti-aging agent (5). Curcumin has significant tumor suppressor potential in a variety of cancers by inhibiting cancer cell proliferation, metastasis, cell cycle entry, or anti-apoptosis. Studies have determined that curcumin is beneficial in regulating oxidative stress, inflammation, and apoptosis in cardiovascular diseases (6, 7). It has been described that curcumin inhibits lung injury and inflammation in APE by microRNA-21/phosphatase and tensin homologue (PTEN) axis and nuclear factor kappaB (NF-κB) pathway (8). In addition, curcumin ameliorates oxidative stress and inhibits apoptosis in diabetic cardiomyopathy (9, 10). Moreover, it can reduce the production of pro-inflammatory cytokines and alleviate myocardial injury (11). It has been validated that curcumin has a protective effect on cardiomyocytes by mediating oxidative stress and apoptotic pathways (12). Curcumin acts against coronary microembolization-induced myocardial damage by which the mechanism is related to reducing myocardial apoptosis and inhibiting inflammation (13). The protective effects of curcumin on cardiovascular diseases have been extensively elucidated. However, the direct effects of curcumin on APE have been poorly studied. MicroRNA-145-5p (miR-145-5p) is cardioprotective in myocardial pathology (14-16). In this research, miR-145-5p was selected as a molecule involved in the mechanism related to curcumin in the process of myocardial injury following APE. IRS1 was found to be a downstream target of miR-145-5p according to bioinformatics analysis. Therefore, it was hypothesized that curcumin alleviates myocardial inflammation, apoptosis, and oxidative stress induced by APE by regulating miR-145-5p.

MATERIALS AND METHODS

Ethical statement

All animal experiments were complied with the ARRIVE guidelines and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval of animal experiments was obtained from the Animal Research Committee of Yantai Qishan Hospital (No. 2019YTY0622).

Pulmonary embolism rat model

Fifty male SD rats (200–250 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. The rats were housed at 24±2°C, with humidity of 50–60%, light and dark cycle of 12 h, and free access to diet and water. An APE rat model was constructed after one week of adaptive feeding. Rats were anesthetized by intraperitoneal injection with 10% chloral hydrate at 400 mg/kg and fixed in a supine position on a constant temperature work table. An intravenous cannula was inserted into the right femoral vein under a stereoscopic microscope, and 12 mg/kg microsphere suspension (40 mg/ml) (Sephadex G-50, diameter 300 µm, Pharmacia Biotech, Freiburg, Germany) was injected into the vena cava (170). For rats in the Sham group, the same procedure was given, but an equal amount of saline was injected intravenously instead of microspheres.

Curcumin is insoluble in water and various plant extracted oils, and slightly soluble in ethanol and glycerol, but it is well dissolved in DMSO, so it is easy to control the drug dose when administered by gavage. The LD50 of DMSO gavage in rats was 28.3 g/kg, which was much higher than the dose in study presented by Wang et al. experiment (18). Therefore, the use of DMSO as an intragastric dose in animal experiments could not cause toxicological and pathological changes in SD rats. Curcumin was dissolved in DMSO to prepare the initial solution, and the mixture of 80% DMSO and 20% ethyl alcohol was used as the gavage agent (19). Curcumin was administered daily (100 mg/kg) for 30 days before modeling (20). One week before modeling, miR-145-5p agomir, agomir NC, miR-145-5p antagonim, antagonim NC (100 nmol/kg once daily for 3 days), sh-IRS1 and sh-NC lentiviral plasmids (200 µL, 2×10^6TU/mL, GenePharma, Shanghai, China) were administered through the caudal vein. Rats were euthanized and the pulmonary artery tissue was collected and stored at 4% paraformaldehyde or –80°C.

Pulmonary artery pressure measurement

At 24 h post-APE surgery, the rat’s right jugular vein was exposed and a 2.7-F microcatheter (internal diameter 0.9 mm; Terumo, Tokyo, Japan) filled with 10 units/ml of heparin in 0.9% saline was entered the right ventricle until a right ventricular pressure waveform was obtained. The catheter was connected to a miniature pressure sensor that recorded and averaged 10 consecutive beats of right ventricular systolic pressure (RVSP) via the PowerLab Data Acquisition System (AD Instruments, Colorado Springs, CO, USA) (21).

Hematoxylin and eosin staining

Pulmonary artery and myocardial tissues were fixed with 4% paraformaldehyde. After alcohol gradient dehydration, the sample was embedded in paraffin and cut into 4 µm slices for H&E dyeing (Beyotime, Shanghai, China). After staining, the sample was sealed and viewed under a microscope.

TUNEL staining

Apoptotic cells in myocardial tissue were measured using the one-step TUNEL Apoptosis Assay kit (Roche, Basel, Switzerland). Paraffin sections after hydration and protease K treatment (20 min) were detected with fluorescently labeled dUTP solution and TdT enzymes at 37°C for 1 hour. Incubation with DNease 1 at 25°C for 10 min was considered as a positive control and dUTP treatment was regarded as a negative control. The sections were treated with DAB for preliminary visualization and then stained with DAPI (KgaA; Sigma-Aldrich, Merck, St. Louis, MO, USA), followed by gradient ethanol dehydration, xylene permeabilization, and neutral balsam sealing. The images were observed using a microscope (LSM700, Carl Zeiss, Oberkochen, Germany).

Immunohistochemical staining

After dehydrating and hydration, the sections were sealed with 3% H2O2. The sections were processed with 10% normal goat serum (No. 16210072; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and incubated with primary antibodies CyPA and CD147 overnight. HRP-conjugated goat anti-rabbit secondary antibody (1:400; A32731; Invitrogen, Carlsbad, CA, USA) was then incubated together, DAB was added for color development, and microscopic images were observed. The percentage of stained area represents the ratio of the complete total area stained to the total tissue.

Biochemical index detection

IL-1β, IL-6, and TNF-α in cardiac tissue were measured using ELISA kits as requested by the manufacturer.

RT-qPCR

Total RNA was separated using the TRizol RNA separation System (Life Technologies, Hewlett, NY, USA) and reverse-
transcribed into cDNA using reverse transcription system kits (Promega, Madison, WI, USA). RT-qPCR was executed using ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). Table 1 presents the sequences of PCR primers. Expression levels were calculated by 2^(-ΔΔCT).

**Western blot**

Myocardial lysates were analyzed by enhanced radioimmunoprecipitation with a lysis buffer (Boster, Wuhan, China) containing a protease inhibitor. A BCA protein assay kit (Boster, Wuhan, China) was utilized to determine the protein concentration. Subsequently, the proteins were isolated using a 10% SDS-PAGE gel and transferred to a PVDF membrane, which was sealed with 5% BSA at room temperature for 1 hour and incubated with diluted primary antibodies p-p65, cleaved caspase-3, nuclear factor erythroid 2-related factor 2 (Nrf2), and HO-1 at 4°C overnight and a goat anti-rabbit secondary antibody labeled with HRP (goat anti-rabbit, ab205718, 1:10,000; goat anti-mouse, ab205719, 1:10000) at room temperature for 1 hour. The protein bands were detected by an ECL kit (EMD, Millipore, MA, USA) and evaluated by Image J (NIH, Bethesda, MD, USA).

**Luciferase reporter activity**

The IRS1 3'-UTR primer containing presumed miR-145-5p targets was amplified by PCR from human genomic DNA, and the DNA fragment was cloned into the pmir-RB-REPORT vector (Ribobio, Guangzhou, China) and named IRS1-WT. For the pmir-RB-IRS1-3'-UTR-MUT vector, the miR-145-5p target site in IRS1-3'-UTR was mutated by PCR-based method. The luciferase reporter vector and miR-145-5p mimic and mimic NC (GenePharma, Shanghai, China) were co-transfected into HEK293 cells using lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) and assayed for luciferase activity 24 h later (E2920, Promega, Madison, WI, USA).

**RNA immunoprecipitation experiment**

RNA immunoprecipitation (RIP) assay was carried out using the EZ-Magna RIP kit (Millipore, Bedford, MA, USA). HEK293 cells were lysed in a complete RIP lysis buffer, and an equal volume of cell extract (100 µL) was incubated with magnetic beads coupled with mouse anti-AgO2 or mouse IgG in RIP buffer. RNA enrichment was analyzed by RT-qPCR.

**Statistical analysis**

All statistical analysis was performed using GraphPad Prism 9 software (GraphPad Software, Boston, MA, USA). Data are expressed as mean ±SD. A two-tailed paired Student’s t-test was used to evaluate the statistical differences for comparisons between two groups, and one-way analysis of variance (ANOVA) with Tukey’s multiple comparisons test was used for comparisons of more than two groups. Multiple comparison of the two independent variables was performed using two-factor ANOVA and Sidak’s multiple comparison test. Specific details for the n value are noted in each figure legend. P<0.05 was considered statistically significant.

**RESULTS**

Curcumin improves myocardial injury induced by pulmonary embolism

APE rat model was established and curcumin was pretreated. HE staining showed that curcumin pretreatment degraded microspheres, inhibited thrombosis, and reduced peripulmonary artery edema (Fig 1A). Hemodynamic tests showed that curcumin pretreatment reduced RVSP in rats (Fig. 1B). Subsequently, the pathological changes of myocardial tissue were examined. HE staining showed that the arrangement of cardiomyocytes in APE rats was disordered and edema existed in the cells, but curcumin pretreatment made the arrangement of cardiomyocytes disordered and edema decreased (Fig. 1C). APE caused an increase in TUNLE-positive cells in the rat myocardium, while curcumin pretreatment decreased the proportion of TUNEL-positive cells (Fig. 1D).

Curcumin ameliorates pulmonary embolism-induced myocardial inflammation

Biochemical tests showed that TNF-α, IL-1β and IL-6 were increased in the myocardial tissue of APE rats. Curcumin pretreatment attenuated these changes in inflammatory and oxidative stress-related factors (Fig. 2A). CyPA-CD147 interaction is involved in myocardial inflammation due to APE (22). IHC staining presented that APE promoted CyPA and CD147 expression, but curcumin pretreatment reduced CyPA and CD147 expression (Fig. 2B). Western blot showed that APE increased p-p65 and cleaved caspase-3 and inhibited Nrf2 and HO-1 protein levels in myocardial tissue, but curcumin pretreatment inhibited the effect of APE (Fig. 2C, and 2D).

**Table 1. Primer sequences.**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequences (5'–3')</th>
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<tbody>
<tr>
<td>miR-370-5p</td>
<td>Forward: 5'−GGTCCAGTTTCCCCAGGA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'TGTTGTCTGGAGGATCG-3'</td>
</tr>
<tr>
<td>IRS1</td>
<td>Forward: 5'-CATCTGCAGAGGAGTGA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CACCATGTCTGGTTGC-3'</td>
</tr>
<tr>
<td>U6</td>
<td>Forward: 5'-CTGCACGCCAGACAGA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-AACGCTCAAAATCTGTC-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward: 5'-CACCCACTTCCTCCACCTTTG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CCACCCACTTGTGGTAG-3'</td>
</tr>
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miR-370-5p, microRNA-370-5p; IRS1, insulin receptor substrate 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
miR-145-5p improves acute pulmonary embolism-induced myocardial injury

APE treatment inhibited miR-145-5p expression (Fig. 3A). miR-145-5p agomir was injected into rats to upregulate miR-145-5p (Fig. 3B). HE staining of the pulmonary artery showed that miR-145-5p overexpression could reduce the aggregation of pulmonary artery particles and reduce the edema around the pulmonary artery (Fig. 3C). The hemodynamic assessment showed that miR-145-5p overexpression reduced RVSP in APE rats (Fig. 3D). Pathological tissue staining manifested that miR-145-5p overexpression reduced myocardial tissue damage (Fig. 3E).

miR-145-5p ameliorates acute pulmonary embolism-induced myocardial inflammation

TUNEL assay showed that overexpression of miR-145-5p significantly reduced the proportion of TUNEL-positive cells in rats (Fig. 4A). IHC staining presented that over-expressing miR-145-5p effectively reduced the number of positive cells for CyPA and CD147 (Fig. 4C). Also, overexpressing miR-145-5p decreased TNF-α, IL-1β and IL-6 in APE rats (Fig. 4B). Western blot discovered that up-regulating miR-145-5p inhibited p-p65 and cleaved caspase-3, and promoted Nrf2 and HO-1 levels (Fig. 4D).

Fig. 1. Curcumin improves myocardial injury induced by APE. (A): HE staining to determine lung histopathology; (B): Determination of RVSP; (C): HE staining to determine myocardial histopathology; (D): TUNEL to determine the effect of curcumin on cardiomyocyte apoptosis. Data are expressed as mean ±SD (n=5). *P<0.05.
Fig. 2. Curcumin ameliorates PE-induced myocardial inflammation. (A): ELISA for TNF-α, IL-1β and IL-6 production; (B): IHC staining to determine the effect of curcumin on CyPA and CD147 expression; (C): Western blot for protein bar graphs of cleaved caspase-3, p-p65, Nrf2 and HO-1 in myocardial tissues; (D): Quantitative bar graph of protein expression of cleaved caspase-3, p-p65, Nrf2 and HO-1 in myocardial tissues. Data are expressed as mean ±SD (n=5). *P<0.05.
Fig. 3. miR-145-5p improves APE-induced myocardial injury. Rats were injected in vivo with miR-145-5p agomir and agomir NC. (A): RT-qPCR to determine the expression of miR-145-5p in rats; (B): RT-qPCR to determine the expression of miR-145-5p in rats after knockdown of miR-145-5p; (C): HE staining to determine the histopathology of the lungs; (D): RVSP determination; (E): HE staining to determine myocardial histopathology. Data are expressed as mean ±SD (n=5). *P<0.01.
Fig. 4. miR-145-5p ameliorates APE-induced myocardial inflammation. Rats were injected with miR-145-5p agomir and agomir NC. (A): TUNEL to determine the effect of up-regulated miR-145-5p on cardiomyocyte apoptosis; (B): ELISA to detect the production of TNF-α, IL-1β, and IL-6; (C): IHC staining to determine the expression of CyPA and CD147; (D): Western blot to detect the protein expression of cleaved caspase-3, p-p65, Nrf2 and HO-1 in myocardial tissues. Data are expressed as mean ±SD (n=5). *P<0.05.
Curcumin improves acute pulmonary embolism-induced myocardial injury by upregulating miR-145-5p

Subsequently, it was investigated whether miR-145-5p was involved in the process of curcumin treating myocardial injury.

RT-qPCR showed that curcumin pretreatment forced miR-145-5p expression in the myocardium of APE rats (Fig. 5A). miR-145-5p antagomir was treated with curcumin pretreatment. Fig. 5B shows that curcumin pretreatment reduced pulmonary artery microsphere aggregation and thrombosis, but this effect was
Fig. 6. Curcumin ameliorates APE-induced myocardial inflammation by upregulating miR-145-5p. Rats were injected with miR-145-5p anta and anta NC. (A): TUNEL to determine the effect of curcumin on the down-regulation of miR-145-5p on apoptosis in cardiomyocytes; (B): ELISA to detect the production of TNF-α, IL-1β, and IL-6; (C): IHC staining to determine the expression of CyPA and CD147; (D): Western blot to detect the protein expression of cleaved caspase-3, p-p65, Nrf2 and HO-1 in myocardial tissues. Data are expressed as mean ±SD (n=5). *P<0.05.
Insulin receptor substrate 1 is mediated by miR-145-5p

Potential binding sites for IRS1 and miR-145-5p were predicted by the bioinformatics website starbase (Fig. 7A). Their targeting relationships were examined by dual luciferase reporting assay and RIP assay. Co-transfection of WT-IRS1 and miR-145-5p mimic reduced luciferase activity (Fig. 7B). IRS1 and miR-145-5p were found to be highly enriched in Ago2 magnetic beads (Fig. 7C). IRS1 was abnormally highly expressed in APE rats, but curcumin pretreatment or miR-145-5p overexpression could reduce this expression pattern in APE rats (Fig. 7D).

Lowering insulin receptor substrate 1 can reduce myocardial injury in acute pulmonary embolism rats

Subsequently, IRS1 was knocked down in APE rats (Fig. 8A). Knocking down IRS1 significantly reduced pulmonary embolism (Fig. 8B) and RVSP in APE rats (Fig. 8C).
Pathological staining showed that IRS1 knockdown reduced cell damage and reduced the proportion of TUNEL-positive cells in the myocardium (Fig. 8D and 8E).

**Reduction of insulin receptor substrate 1 attenuates myocardial inflammation in acute pulmonary embolism rats**

IHC staining showed that knocking down IRS1 reduced the positive rate of CyPA and CD147 in myocardial tissue (Fig. 9B). Moreover, knocking down IRS1 reduced the release of inflammatory cytokines (Fig. 9A). Western blot manifested that IRS1 knockdown inhibited p-p65 and cleaved caspase-3 expression and forced Nrf2 and HO-1 expression (Fig. 9C).

**Knocking down insulin receptor substrate 1 can enhance the therapeutic effect of curcumin on myocardial injury in acute pulmonary embolism rats**

IRS1 was knocked down while curcumin was pretreated (Fig. 10A). IRS1 knockdown could enhance the degradation
effect of curcumin on microembolism (Fig. 10B). In addition, IRS1 knockdown further enhanced curcumin’s inhibitory effect on RVSP (Fig. 10C). Myocardial pathological staining showed that curcumin pretreatment could reduce myocardial tissue damage and apoptosis rate, while IRS1 knockdown enhanced the effect of curcumin (Fig. 10D and 10E).

Knockdown of insulin receptor substrate 1 enhances the inhibitory effect of curcumin on myocardial inflammation in acute pulmonary embolism rats

Curcumin reduced the release of inflammatory factors, while knockdown IRS1 enhanced this effect of curcumin (Fig. 11A). IHC staining showed that IRS1 knockdown enhanced curcumin inhibition of CyPA and CD147 (Fig. 11B). Western blot showed that curcumin pretreatment inhibited p-p65 and cleaved caspase-3 expression and promoted Nrf2 and HO-1 expression, which was enhanced by knockdown IRS1 (Fig. 11C).

DISCUSSION

APE is a life-threatening diagnosis that can present with a variety of non-specific symptoms. To control APE-induced myocardial injury, this research paid prevalent attention to curcumin regarding its functions and related mechanisms. Ultimately, it was found that curcumin alleviates myocardial inflammation, apoptosis, and oxidative stress induced by APE via the miR-145-5p/IRS1 axis.

Curcumin treatment has shown great therapeutic potential in rats with APE by reducing RVSP, edema, and thrombus volume and managing the production of inflammatory factors in the lungs (8). Previous studies have shown that curcumin reduces lung injury and inflammatory response in APE rats by inhibiting the expression of Sp1 and down-regulating the expression of miR-21, which in turn up-regulates the expression of PTEN and inhibits the NF-κB signaling pathway (8). Meanwhile, curcumin alleviates pneumonia in neonatal rats with lipopolysaccharide-induced acute lung injury by reducing the levels of TNF-α and IL-6 (23). Therefore, it is speculated that curcumin may prevent APE by inhibiting lung injury and inflammatory response. This research also confirmed the protective effect of curcumin against lung injury in APE rats by degrading microspheres, inhibiting thrombosis, and reducing edema and RVSP. Furthermore, studies have observed the functions of curcumin in myocardial injuries. In fact, curcumin protects against myocardial ischemia-reperfusion injury (MI/RI) by reducing inflammation, apoptosis, and oxidative stress (24). Also, another research confirms the

![Graph showing the effect of curcumin on inflammatory factors](image)

**Fig. 9.** Reduction of IRS1 attenuates myocardial inflammation in APE rats. Rats were injected in vivo with sh-IRS1 and sh-NC. (A): ELISA to detect the production of TNF-α, IL-1β, and IL-6; (B): IHC staining to determine the expression of CyPA and CD147; (C): Western blot to detect the protein expression of cleaved caspase-3, p-p65, Nrf2, and HO-1 in myocardial tissue. Data are expressed as mean ±SD (n=5). *P<0.01.
beneficial effect of curcumin through controlling myocardial oxidation, apoptosis, and inflammation indicators in MI/RI (25). It has been demonstrated that curcumin analog C66 attenuates myocardial injury in obese mice by inhibiting inflammation (26). Curcumin restores biochemical indices, maintains antioxidant capacity, and reduces pro-inflammatory cytokine in diabetes-induced myocardial infarction (10). Consistent with these study findings, our research discovered that curcumin pretreatment recovered pathological changes in myocardial tissue and reduced apoptosis, inflammatory responses, and oxidative stress by altering relevant indicators.

In rats suffering from APE, it was found that miR-145-5p expression was lowered, indicating the involvement of miR-145-5p in APE pathogenesis. Based on that, this study further explored the potential function of miR-145-5p in APE and finally demonstrated that miR-145-5p overexpression phenocopied the beneficial effect of curcumin on APE rats in the lung and myocardium. A current report has noted that miR-145-5p is downregulated in acute lung injury rats, and upregulating miR-145-5p attenuates lung injury by relieving pathological damage and edema and reducing apoptosis and inflammatory response (27). Besides, miR-145-5p has been proven to participate in lung injury by lowering pro-inflammatory cytokines and suppressing ROS accumulation (28). In terms of myocardial injury, it has been determined that miR-145-5p overexpression reduces the production of inflammatory, apoptotic, and oxidant indices in animal models with MI/RI (15, 29). In addition, miR-145-5p overexpression has an effective effect on suppressing inflammatory factor production and apoptosis in cardiomyocytes in a hypoxic environment.

**Fig. 10.** Knocking down IRS1 can enhance the therapeutic effect of curcumin on myocardial injury in APE rats. Rats were injected with sh-IRS1 and sh-NC. (A): Western blot to determine the effect of curcumin on the expression of IRS1; (B): HE staining to determine the lung histopathology; (C): RVSP determination; (D): HE staining to determine the myocardial histopathology; (E): TUNEL to determine the effect of curcumin on the apoptosis of cardiomyocytes after down-regulation of IRS1. Data are expressed as mean ±SD (n=5). *P<0.05.
Taking curcumin and miR-145-5p in combination into consideration, the current research proved that the beneficial effect of curcumin as aforementioned on APE rats could be weakened by knocking down miR-145-5p, confirming that its improves APE-induced myocardial injury by upregulating miR-145-5p. Former studies have observed the upregulating effect of curcumin on miR-145 expression in the tumor environment (32, 33).

**Fig. II.** Knockdown of IRS1 enhances the inhibitory effect of curcumin on myocardial inflammation in APE rats. Rats were injected with sh-IRS1 and sh-NC. (A): ELISA to detect the production of TNF-α, IL-1β, and IL-6; (B): IHC staining to determine the expression of CyPA and CD147; (C): Western blot to detect the protein expression of cleaved caspase-3, p-p65, Nrf2, and HO-1 in myocardial tissue. Data are expressed as mean ±SD (n=5). *P<0.05.
IRS1, an identified target of miR-145-5p, was highly expressed in APE rats. Functionally, knocking down IRS1 was effective in alleviating apoptosis, inflammation, and oxidative stress in APE rats. It has been reported that miR-128 targets and negatively regulates the expression of IRS1, thereby improving the functional impairment of cardiac microvascular endothelial cells and reducing oxidative stress in coronary heart disease (34). Notably, androgen can improve insulin resistance in chronic heart failure and reduce IRS1 expression, thereby reducing myocardial apoptosis (35). Meanwhile, an observational report has confirmed that aldehyde dehydrogenase-2 may effectively reduce myocardial remodeling and contractile defects by inactivating IRS1/Akt pathway (36). This research additionally evaluated the combined effect of curcumin and IRS1 and finally reported that knocking down IRS1 can enhance the therapeutic effect of curcumin on myocardial injury in APE rats.

There are also some limitations to this study. Only animal experiments were conducted in this study, and cell experiments should be supplemented in the future to confirm in vivo results. In addition, experimental results need repeated confirmations and investigations before being put into clinical practice. Therefore, the clinical value of this study needs repeated confirmations and investigations.

This study elaborated on the protective properties of curcumin in APE rats in the liver and myocardial injuries. Moreover, this study initially delineated the interplay between curcumin and miR-145-5p/IRS1 targeting axis in APE, highlighting that curcumin upregulates miR-145-5p and downregulates IRS1 to relieve myocardial inflammation, oxidative stress, and apoptosis in APE rats.

Authors’ contribution: J. Ling, W. Li and X.L. Gong designed the research study. G.Y. Wang and F. Zhao performed the research. L. Han provided help and advice. G.Y. Wang, F. Zhao and L. Han analyzed the data. J. Ling, W. Li and X.L. Gong wrote the manuscript. L. Han reviewed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ling Jiang, Wei Li, and XueLian Gong contributed equally to this work.

Availability of data and materials: The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Conflict of interests: None declared.

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