Knockdown of ALOX15 alleviates acute coronary syndrome via the FGFR2/PI3K/AKT signaling pathway

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Abstract. Acute coronary syndrome (ACS) is a serious cardiovascular condition and a leading cause of mortality worldwide. Notably, 12/15-lipoxygenase (ALOX15) can be regulated by the long non-coding RNA ENST00000538705.1, thereby facilitating the progression of ACS. However, the downstream regulatory mechanisms involving ALOX15 remain unclear. The viability and migration of human primary coronary artery endothelial cells (HCAECs) were assessed using the Cell Counting Kit-8 and scratch assays, respectively. Reverse transcription-quantitative PCR was performed to assess the mRNA expression levels of ALOX15 and fibroblast growth factor receptor 2 (FGFR2). Protein-protein interactions between ALOX15 and FGFR2 were verified by co-immunoprecipitation (CO-IP). An ACS rat model was established to examine the effects of ALOX15 on blood lipid levels. Hematoxylin and eosin staining was executed to assess the histological changes. The levels of the FGFR2/PI3K/AKT signaling pathway-related proteins were assessed by western blotting. The results revealed elevated expression levels of ALOX15 and FGFR2 in patients with ACS. In HCAECs, transfection of overexpressed ALOX15 markedly enhanced cell viability and migration, while small interfering RNA-ALOX15 transfection produced the opposite effects. CO-IP assays confirmed the interaction between ALOX15 and FGFR2 in HCAECs. Additionally, knockdown of ALOX15 reduced blood lipid levels and alleviated myocardial injury in rats with ACS. ALOX15 silencing inhibited the expression of proteins associated with the FGFR2/PI3K/AKT signaling pathway in both HCAECs and rats with ACS. Both the overexpression of FGFR2 and the supplementation with insulin like growth factor 1 (a specific agonist of the PI3K/AKT pathway) significantly mitigated the inhibitory effects of ALOX15 knockdown on the migratory and proliferative capacities of HCAECs. The findings of the present study indicated that silencing of ALOX15 alleviates ACS progression via inhibiting the FGFR2/PI3K/AKT signaling pathway, providing a theoretical basis for ACS therapy in clinic.

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Abbreviations: ACS, acute coronary syndrome; HCAECs, human primary coronary artery endothelial cells; CAD, coronary heart disease; STEMI, ST-elevated myocardial infarction; NSTEMI, non-ST elevated myocardial infarction; ALOX15, 12/15-lipoxygenase; HPETE, hydroperoxy-eicosatetraenoic acid; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; CO-IP, co-immunoprecipitation; FGFR, fibroblast growth factor receptor

Key words: acute coronary syndrome, ALOX15, FGFR2/PI3K/AKT pathway, viability, migration

Introduction

Acute coronary syndrome (ACS) is a fatal manifestation of coronary heart disease (CAD) comprising three main types: Unstable angina (UA), ST-elevated myocardial infarction (STEMI), or non-ST elevated myocardial infarction (NSTEMI) (1,2). ACS is primarily caused by plaque rupture, erosion, and thrombosis (3,4), leading to a significant reduction in coronary blood flow (5). A retrospective cohort study conducted by Kaul *et al* (6) found that patients with UA, STEMI and NSTEMI had a high likelihood of developing heart failure (16, 23.4 and 25.4% respectively), suggesting that ACS may be a significant risk factor for the development of heart failure. Currently, although some treatments have been proven effective for patients with ACS in clinical practice, recurrent events and adverse effects, such as hypotension and hyperkalemia, remain often unavoidable (7). Therefore, identifying effective therapies with minimal side-effects to alleviate clinical symptoms and improve the quality of life for patients with ACS is crucial from both medical and socioeconomic perspectives.

Notably, 12/15-lipoxygenase (ALOX15), containing 13 introns and 14 exons, is mapped at LOX gene cluster on chromosome 17 and can encode arachidonic acid 12/15-lipoxygenase (12/15-LOX) (8). It is acknowledged

that 12/15-LOX is a type of dioxygenase enzyme, which is crucial in incorporating oxygen into polyunsaturated fatty acids to generate biologically-active peroxide products such as 13-hydroperoxy-octadecadienoic acid and hydroperoxy-eicosatetraenoic acid (HPETE) (8). Reports on ALOX15 in ACS are currently extremely limited. Research on ALOX15 in cardiac disorders has mainly focused on myocardial ischemia-reperfusion injury. Previous studies have demonstrated that 12/15-LOX plays an essential role in inducing the transformation of low-density lipoprotein (LDL) into atherosclerotic lesions, indicating that ALOX15 may possess proatherogenic properties (9-11). Ma *et al* (12) found that ALOX15-induced peroxidation of polyunsaturated fatty acid-phospholipids increases the susceptibility to ferroptosis in ischemia-induced myocardial damage (12). Similarly, Cai *et al* (13) proposed that ALOX15/HPETE-mediated cardiomyocyte ferroptosis plays a crucial role in prolonged myocardial ischemia-reperfusion injury (13). Additionally, ALOX15 activation-induced inflammation has been confirmed to be associated with the development of heart failure (14). As early as 2013, Silbiger *et al* (15) conducted a transcriptional profiling analysis and indicated that ALOX15 is one of the most significant gene expression biomarkers for the very early stages of ACS (15). Notably, our previous study indicated that ALOX15 can be regulated by the long non-coding RNA ENST00000538705.1 to promote progression of ACS (16), however the downstream regulatory mechanisms of ALOX15 remain unclear.

Phosphoinositide 3-kinases (PI3Ks) and their downstream target serine/threonine kinase AKT (also known as protein kinase B) are a conserved family of signal transduction enzymes (17). Previous research has increasingly revealed the crucial role of the PI3K/AKT signaling pathway in cardiac disorders. For example, the resveratrol-mediated activation of the PI3K/AKT pathway exerts cardioprotection by reducing mitochondrial oxidative damage during myocardial ischemia/reperfusion injury (18). By modulating the PI3K/AKT pathway, Qingda granule can attenuate angiotensin II-induced cardiac hypertrophy and apoptosis (19). In a mouse model of myocardial infarction or heart failure, activation of the PI3K/AKT pathway has been shown to promote angiogenesis and provide cardioprotection (20,21). However, whether the PI3K/AKT pathway plays a regulatory role in ACS is still in the preliminary stage of exploration. Fibroblast growth factor receptors (FGFRs), belonging to the transmembrane tyrosine kinase receptor family, are strongly associated with multiple cellular and biological processes such as cell growth, migration, angiogenesis and wound repairment (22,23). Currently, four main types of FGFRs have been identified, including FGFR1, FGFR2, FGFR3 and FGFR4 (24,25). Among these subtypes, FGFR2 plays a particularly essential role in cell differentiation, growth and migration (26). It is widely reported that endothelial dysfunction is strongly associated with the progression of ACS (27). A recent study conducted by Jiao *et al* (28) demonstrated that FGFR2 mediates endothelial dysfunction by regulating the AKT/Nrf2/ARE signaling pathway. Furthermore, inhibition of FGFR2 has been proposed as therapeutic target for cardiac fibrosis (a hallmark pathological feature following myocardial injury) (29). Notably, FGFR2 functioning as an activator of the PI3K/AKT pathway has been widely reported in numerous human diseases (30,31). Currently, there are no relevant research studies on the interaction between ALOX15 and the FGFR2/PI3K/AKT signaling pathway in the progression of ACS. Therefore, determining whether the FGFR2/PI3K/AKT pathway, mediated by ALOX15, exerts regulatory functions in the progression of ACS has sparked our great interest.

In the present study, the effects of ALOX15 on human primary coronary artery endothelial cells (HCAECs) were investigated. In addition, a rat model of ACS was established. The aim of the study was to preliminarily explore the downstream regulatory mechanism of ALOX15 in ACS pathology.

Materials and methods

Patients. A total of 30 patients with ACS (15 women and 15 men; age range, 52-70 years; mean age, 60.5±6.4 years) were admitted to The Third Affiliated Hospital of Shanghai University (Wenzhou People's Hospital; Wenzhou, China). The inclusion criteria were as follows: i) Diagnosis of UA, STEMI or NSTEMI; ii) postoperative blood flow of the target vessel reaching thrombolysis in myocardial infarction (TIMI) grade; iii) complete clinical history available; iv) no surgical contraindications. The exclusion criteria were as follows: i) Presence of congenital heart disease, heart failure, or peripheral vascular diseases; ii) history of prior cardiac surgery, such as coronary stent implantation or coronary artery bypass; iii) recent history of infectious diseases; iv) missing or incomplete case data, or lack of cooperation with follow-up. Concurrently, 30 healthy controls (15 women and 15 men; age range, 48-66 years; mean age, 56.7±6.4 years) were selected as the physical examination population, with similar age and sex to the experimental group, no symptoms of chest tightness and chest pain, and no obvious abnormalities in biochemical tests, electrocardiogram, chest CT, cardiac color Doppler ultrasound and other imaging tests. Venous blood samples from patients with ACS and healthy controls were collected, centrifuged at 3,000 x g at 4˚C for 5 min, and then stored at -80˚C for subsequent experiments. The patients/participants provided their written informed consent to participate in this study. The present study was conducted according to the guidelines of the Declaration of Helsinki and approved (approval no. 2020-351) by the Ethics Committee of The Third Affiliated Hospital of Shanghai University (Wenzhou People's Hospital; Wenzhou, China).

Animals, cells and reagent. A total of 18 male Sprague-Dawley rats weighing 250-280 g (8 weeks old) was obtained from Vital River Laboratory Animal Technology Co., Ltd. HCAECs (passage 2; cat. no. CP-H087) and rat coronary artery endothelial cells (cat. no. CP-R081) were purchased from Procell Life Science & Technology Co., Ltd. Approval for the use of primary cells was obtained from the Ethics Committee of The Third Affiliated Hospital of Shanghai University (Wenzhou People's Hospital; Wenzhou, China). 293 cells were obtained from Thermo Fisher Scientific, Inc. Endothelial basal medium 2 (EBM-2) and fetal bovine serum (FBS) were purchased from Lonza Group, Ltd. The Cell Counting Kit-8 was provided by Abbkine Scientific Co., Ltd. Small hairpin targeting ALOX15 (shRNA ALOX15),

Table I. Reverse transcription quantitative-PCR primer synthesis list.

Gene	Sequences	
ALOX15	Forward	5'-CCGCTGCTGTTTGTGAAACT-3'
	Reverse	5'-AGCGGTAACAAGGGAACCTG-3'
FGFR2	Forward	5'-TGACCAAACGTATCCCCCTG-3'
	Reverse	5'-GGTGTCTGCCGTTGAAGAGA-3'
GAPDH	Forward	5'-TCAAGAAGGTGGTGAAGCAGG-3'
	Reverse	5'-TCAAAGGTGGAGGAGTGGGT-3'

ALOX15, 12/15-lipoxygenase; FGFR2, fibroblast growth factor receptor 2.

negative control (shRNA NC), small interfering RNA (siRNA)1/2/3 ALOX15 along with their corresponding negative control (siRNA NC), as well as pcDNA3.1-ALOX15 (OE-ALOX15), OE-FGFR2 and the pcDNA3.1 empty vector (OE-NC) were synthesized by Genomeditech (Shanghai) Co., Ltd. The immunoprecipitation kit with protein A+G magnetic beads (cat. no. P2179S), hematoxylin and eosin (H&E) staining kit (cat. no. C0105S), insulin-like growth factor 1 (IGF-1; cat. no. P5502), RIPA lysis buffer (cat. no. P0013B), ECL kit (cat. no. P0018M) and BCA kit (cat. no. P0012) were obtained from Beyotime Insitute of Biotechnology. Guangzhou RiboBio Co., Ltd. supplied the riboFECT™ mRNA transfection agent (cat. no. C11055-1). Lipofectamine 3000 (cat. no. L3000150) was obtained from Thermo Fisher Scientific, Inc. The total cholesterol (TC) content assay kit (cat. no. BC1980) was acquired from Beijing Solarbio Science & Technology Co., Ltd., while the high-density lipoprotein-cholesterol (HDL-C) assay kit (cat. no. A112-1-1) and low-density lipoprotein-cholesterol (LDL-C) assay kit (cat. no. A113-1-1) were sourced from Nanjing Jiancheng Bioengineering Institute. TRIzol reagent (cat. no. 10606ES60), Hifair® II 1st Strand cDNA Synthesis SuperMix (cat. no. 11123ES60) and Hieff® qPCR SYBR Green Master Mix (cat. no. 11202ES08) were procured from Shanghai Yeasen Biotechnology Co., Ltd. Primary antibodies AKT (cat. no. 60203-2-Ig), phoshorylated (p)-AKT (cat. no. 66444-1-Ig), GAPDH (cat. no. 60004-1-Ig) and HRP-conjugated secondary antibodies (cat. no. SA00001-2) were obtained from Proteintech Group, Inc. Primary antibodies p-PI3K (cat. no. 4228T) and FGFR2 (cat. no. 23328) were purchased from Cell Signaling Technology, Inc., and PI3K antibody (cat. no. ab191606) was sourced from Abcam. ALOX15 antibody (cat. no. sc-133085) was obtained from Santa Cruz Biotechnology, Inc.

Cell culture. HCAECs were cultured in EBM-2 containing 15% FBS. The culture cells were maintained in an environment with 5% CO_2 and a temperature of 37˚C. Cells in the logarithmic growth phase were harvested for functional experiments.

Cell transfection and treatment. Cell transfection experiments were conducted in strict compliance with the protocols of the riboFECT™ mRNA transfection agent. Briefly, OE-ALOX15, OE-FGFR2, siRNA1/2/3 ALOX15 and their corresponding NCs (all, 50 nM) were individually introduced into HCAECs using the riboFECT™ mRNA transfection agent for 48 h at 37˚C. Following a 48-h period, the cells were collected for the subsequent experiments. The sequences of siRNA1/2/3 ALOX15 and siRNA NC were as follows: siRNA1 ALOX15 forward, 5'-GUCGAUACAUCCUAUCUUCAATT-3' and reverse, 5'-UUGAAGAUAGGAUGUAUCGACTT-3'; siRNA2 ALOX15 forward, 5'-AUGACUUCAACCGGAUUUUCUTT-3' and reverse, 5'-AGAAAAUCCGGUUGAAGUCAUTT-3'; siRNA3 ALOX15 forward: 5'-CGCUAUCAAAGACUCUCUAAATT-3' and reverse, 5'-UUUAGAGAGUCUUUGAUAGCGTT-3'; siRNA NC forward, 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse: 5'-ACGUGACACGUUCGGAGAATT-3'. Additionally, to ascertain the mutual effect between ALOX15 and the PI3K/AKT pathway through reverse transcription-quantitative PCR (RT-qPCR), scratch tests, CCK-8 assay and western blot analysis, 4 μl of PI3K/AKT pathway specific agonist IGF-1 was added to HCAECs for 24 h at 37˚C.

RT-qPCR. The extraction of RNA was conducted with the aid of TRIzol reagent. The concentration of total RNA was measured using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Total RNA (500 ng) was reversible transcribed into cDNA via Hifair® II 1st Strand cDNA Synthesis SuperMix at 45˚C for 45 min. Subsequently, PCR analysis was carried out in accordance with the protocols of Hieff® qPCR SYBR Green Master Mix. The reaction conditions were as follows: One cycle at 95˚C lasting 2 min, 40 cycles at 95˚C for 10 sec, 60˚C for 30 sec, and 72˚C for 30 sec. The primers used in the present study are listed in Table I. Relative mRNA expression of ALOX15 and FGFR2 was normalized against GAPDH and calculated with the $2^{-\Delta\Delta Cq}$ method (32).

Scratch tests. The transfected HCAECs were seeded in 6-well plates at a density of $3x10^5$ cells/well. Upon reaching 100% confluence, a scratch was created in the monolayer using a pipette tip (10 μl), and HCAECs were then incubated in a serum-free medium for 48 h. The width of the scratches at both 0 h and 48 h was recorded under a light microscope (Olympus Corporation). The migratory abilities of HCAECs were analyzed with ImageTool software version 1.46 (University of Texas Health Science Center at San Antonio), employing the following formula: (The width at 0 h - the width at 48 h)/the width at 0 h x 100%.

CCK-8 assay. HCAECs were inoculated into culture plates with 96-wells ($4x10^3$ cells/well) and subsequently cultured for 24, 36, 48, and 72 h, respectively. A total of 10 μl of CCK-8 solution was added into each well and the cells were then incubated for 2 h at 37˚C. Cell viability was then assessed using a microplate reader (DR-3518G, Wuxi Hiwell Diatek Instruments, Co., Ltd.) at an absorbance of 450 nm.

Co-immunoprecipitation (CO-IP) assay. For per IP reaction, 1 ml of lysis buffer was added to HCAECs ($2x10^7$ cells) and incubated on ice for 30 min. Following centrifugation at 14,000 x g for 20 min at 4˚C, the cell lysates from HCAECs were collected, and the resulting supernatants were incubated with 40 μl of a 1:1 slurry of Protein G-Sepharose beads conjugated to rabbit anti-ALOX15 (5 μg) and anti-FGFR2 (5 μg) antibodies at 4˚C overnight. Concurrently, an anti-IgG antibody (5 μg) was used as a control. The beads were then washed 5 times with 1 ml wash buffer and centrifuged at 1,000 x g for 1 min at 4˚C between washes. After the final wash, the supernatant was removed, and 30 μl of elution buffer was added in the samples and boiled at 95˚C for 5 min. Subsequently, the samples were subjected to gradient SDS-polyacrylamide gels and transferred to membranes. The immunoprecipitates obtained were then analyzed by western blotting.

Animal grouping and treatment. The ACS rat model was established based on previously described protocols (33,34). Approval for the use of animals was obtained from the Ethics Committee of Wenzhou Medical University (Wenzhou, China). In these previous studies, the modeling method for ACS closely resembled that of acute myocardial infarction, as the latter is one of the clinical manifestations of ACS. Sprague-Dawley rats were randomly assigned to three groups: The sham group, the ACS model + shRNA NC group, and the ACS model + shRNA ALOX15 group, with six rats in each group. The rats were intraperitoneally injected with 50 mg/kg of pentobarbital sodium for anesthesia. Their limbs were then secured to a surgical table while maintaining them in a supine position. Following thoracotomy, the heart was exposed and the anterior descending coronary artery was ligated using 5/0 surgical sutures. To prevent infection, penicillin (100,000 IU) was subcutaneously injected into each rat. Meanwhile, only threading was performed in rats in the sham group, without ligation. The electrocardiogram results were recorded and the successfully established ACS rat model was indicated as follows: ST segment and/or high T wave was markedly elevated. A 3rd-generation system was used and 9 μg lentiviral vectors were co-transfected into the 293 cells through Lipofectamine 3000 at room temperature. The rate of lentiviral plasmid: packaging vector: envelope was 1:1:1. Lentivirus-containing medium was collected 48 h after transfection and used to infect rat coronary artery endothelial cells (passage 2; $5x10^6$ cells/well) at a multiplicity of infection of 20. Subsequently, 48 h after lentiviral infection, 1 μg/ml puromycin was used to select the infected cells. After 72 h, rats in the ACS model + shRNA NC group received an intramyocardial injection of shRNA NC (5'-GCTGTT CGATCGGGAAACA-3') integrated into lentiviral vectors (50 μg), and those in the ACS model + shRNA ALOX15 group were treated with lentivirus containing shRNA ALOX15 sequence (5'-GCTGATGCCTGATGGACAA-3') via intramyocardial injection. After 2 weeks, the rats in the different groups were euthanized with an overdose of pentobarbital sodium (200 mg/kg) via intraperitoneal injection. Whole blood samples (from the eyeballs) and heart were collected for subsequent tests. Animal experiments were conducted in compliance with the Guidelines for the Use of Laboratory Animals and approved (approval no. xmsq2023-1367) by the Ethics Committee of Wenzhou Medical University (Wenzhou, China).

Biochemical tests. The whole blood samples obtained from the eyeballs of the rats were placed at room temperature for 2 h before being centrifuged at 1,000 x g for 20 min at room temperature. Commercial Kits were employed to determine the levels of TC, HDL-C and LDL-C in serum using a fully automatic bio analysis machine.

H&E staining. Rat myocardial tissues were fixed in 4% paraformaldehyde, deparaffinized and hydrated prior to paraffin embedding. Subsequently, the tissues were sectioned into 4-μm thick slices. The sections were stained with H&E for 3 min. Following mounting with neutral gum, images were captured using an optical microscope (OLYMPUS BX53; Olympus Corporation).

Western blot analysis. Total proteins were extracted from HCAECs or myocardial tissues using RIPA lysis buffer and then measured the concentration with a BCA Kit. All steps were conducted on ice. Proteins (50 μg protein/lane) were then separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane. The membrane was blocked using 5% nonfat milk-Tris-buffered saline with 0.05% Tween-20 for 2 h at 25˚C, on which primary antibodies (ALOX15, 1:1,000; FGFR2, 1:1,000; PI3K, 1:1,000; p-PI3K, 1:1,000; AKT, 1:5,000; p-AKT, 1:2,000; GAPDH, 1:5,0000) were incubated overnight at 4˚C. Subsequently, the corresponding secondary antibodies (1:1,000) were added followed by incubation for 1 h at 37˚C. GAPDH was the normalization for proteins. Protein signals were detected using an ECL kit under Gel-Pro analyzer (version 4.0; Media Cybernetics, Inc.).

Statistical analysis. *In vitro* experiments were performed in triplicate, and each experiment was repeated 3 times. *In vivo* experiments were performed using 6 rats per group. Unpaired Student's t-test was adopted to gauge the differences between two groups. The variations among multiple groups were gauged by applying one-way ANOVA, followed by Tukey's post hoc multiple comparisons test. Data analysis was performed using SPSS software v22.0 (IBM Corp.). The data were indicated as the mean ± standard deviation. $P<0.05$ was considered to indicate a statistically significant difference.

Results

High expression of ALOX15 and FGFR2 is observed in the serum of patients with ACS. After obtaining informed consent from the patients, blood samples were collected from individuals diagnosed with ACS. Following centrifugation,

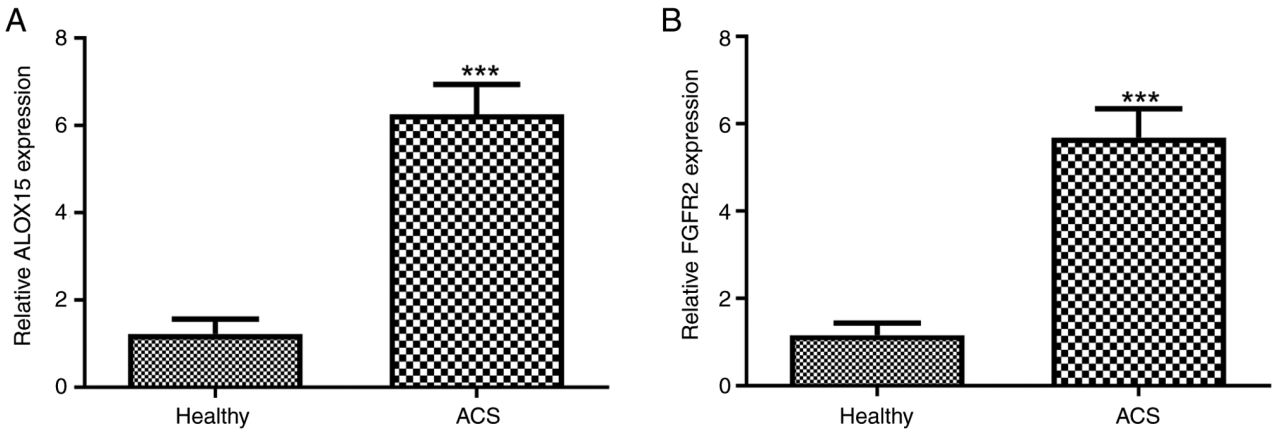

Figure 1. High expression of ALOX15 and FGFR2 is observed in the serum of patients with ACS. The expression of (A) ALOX15 and (B) FGFR2 in patients with ACS and healthy individuals was detected by reverse transcription-quantitative PCR. ***P<0.001 vs. Healthy. ALOX15, 12/15-lipoxygenase; FGFR2, fibroblast growth factor receptor 2; ACS, acute coronary syndrome.

the expression levels of ALOX15 and FGFR2 in the serum of patients with ACS were assessed. As illustrated in Fig. 1A, a significant upregulation of ALOX15 expression in patients with ACS compared with healthy individuals (P<0.001) was observed. Similarly, elevated levels of FGFR2 were also detected in patients with ACS when compared with healthy controls (Fig. 1B; P<0.001).

Effects of ALOX15 on ACS progression in vitro. ALOX15 was then overexpressed or silenced to evaluate its effects on the progression of ACS *in vitro*. Following OE-ALOX15 transfection, the expression of ALOX15 in HCAECs was significantly increased (Fig. 2A; P<0.001). Meanwhile, siRNA1 ALOX15, siRNA2 ALOX15 and siRNA3 ALOX15 were individually transfected into HCAECs. As revealed in Fig. 2B, the expression levels of ALOX15 were significantly reduced after siRNA ALOX15 transfection (P<0.001). Notably, siRNA3 ALOX15 was selected for the subsequent experiments due to its relatively high transfection efficiency. Wound healing assays were then performed and it was revealed that the overexpressed ALOX15 significantly increased the migratory abilities of HCAECs; conversely, silenced ALOX15 exhibited an inhibitory effect on migration in these cells (Fig. 2C and D; P<0.01). Moreover, CCK-8 asays showed that the proliferative ability of HCAECs were increased following OE-ALOX15 transfection, but attenuated after siRNA ALOX15 treatment, in a time-dependent manner (Fig. 2E; P<0.01).

Effects of ALOX15 on the FGFR2/PI3K/AKT signaling pathway in HCAECs. CO-IP analysis demonstrated that ALOX15 protein could interact with FGFR2 in HCAECs (Fig. 3A). Furthermore, following transfection, it was demonstrated that the mRNA expression of ALOX15 and FGFR2 was significantly upregulated by the ectopic expression of ALOX15, while silencing of ALOX15 resulted in a significant decrease in their expression (Fig. 3B; P<0.001). Additionally, the effects of ALOX15 overexpression or silencing on the levels of FGFR2/PI3K/AKT signaling pathway-related proteins were also examined via western blotting. As illustrated in Fig. 3C, the results indicated that in HCAECs, the protein levels of ALOX15, FGFR2, p-PI3K and p-AKT were all markedly enhanced after OE-ALOX15 transfection, while they were reduced after transfection with siRNA ALOX15 (P<0.001). Notably, the protein levels of PI3K and AKT appeared unchanged following treatment with OE-ALOX15 or siRNA ALOX15.

Silencing of ALOX15 mitigates ACS progression in vitro through the FGFR2/PI3K/AKT signaling pathway. Firstly, FGFR2 was overexpressed in HCAECs to examine the interaction of ALOX15 with FGFR2 (P<0.001), as depicted in Fig. 4A. Meanwhile, 4 μl of PI3K/AKT pathway specific agonist IGF-1 was added to HCAECs to further ascertain the mutual effect between ALOX15 and the PI3K/AKT pathway. As illustrated in Fig. 4B, it was determined that both the overexpression of FGFR2 and the addition of IGF-1 reversed the suppressive effects of ALOX15 knockdown on the mRNA expression of ALOX15 and FGFR2 (P<0.05). Similarly, the inhibition of the PI3K/AKT pathway induced by ALOX15 knockdown was also partially counteracted following the addition of OE-FGFR2 or IGF-1 (Fig. 4C; P<0.001). Additionally, it was further demonstrated that both the overexpression of FGFR2 and the addition of IGF-1 significantly mitigated the inhibitory effects of ALOX15 knockdown on the migratory and proliferative abilities of HCAECs (Fig. 4D-F; P<0.05).

Knockdown of ALOX15 reduces serum lipid levels and improves myocardial injury in an ACS rat model. An ACS rat model was subsequently established. As shown in Fig. 5A and B, a significant elevation in the mRNA expression levels of ALOX15 and FGFR2 in rats subjected to ACS (P<0.001) was observed, while injection of shRNA ALOX15 reversed the increase of ALOX15 and FGFR2 levels induced by the ACS challenge (P<0.01). Compared with the rats in the sham group, the serum levels of TC and LDL-C were significantly increased in the rats with ACS, while HDL-C levels were significantly decreased (Fig. 5C; P<0.01). Notably, intramyocardial injection of shRNA ALOX15 not only reduced the levels of TC and LDL-C, but also increased the levels of HDL-C in the rats with ACS (Fig. 5C; P<0.05). Subsequently, the heart tissues from different groups of rats were collected for pathological examination. As illustrated in Fig. 5D, cardiomyocytes

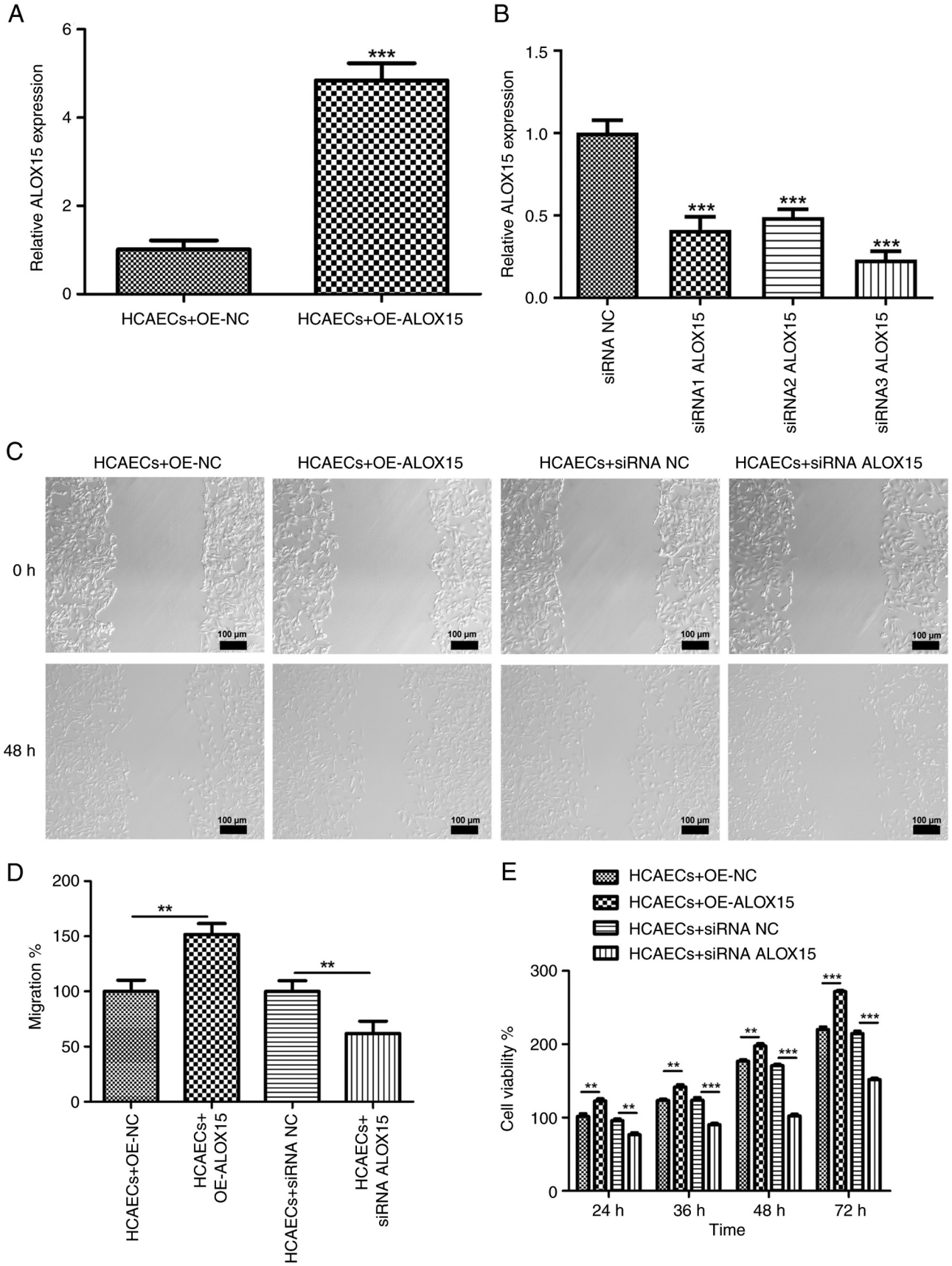

Figure 2. Effects of ALOX15 on acute coronary syndrome progression *in vitro*. (A) The expression of ALOX15 in HCAECs after transfection with OE-ALOX15 or OE-NC was detected by RT-qPCR. ***P<0.001 vs. HCAECs + OE-NC. (B) The expression of ALOX15 in HCAECs after transfection with siRNA1 ALOX15, siRNA2 ALOX15, siRNA3 ALOX15 or siRNA NC was detected by RT-qPCR. ***P<0.001 vs. siRNA NC. (C and D) The migration of HCAECs transfected with OE-ALOX15/OE-NC or siRNA ALOX15/siRNA NC was measured by scratch wound healing assays. **P<0.01. Scale bar, 100 μm. (E) The viability of HCAECs transfected with OE-ALOX15/OE-NC or siRNA ALOX15/siRNA NC was measured by Cell Counting Kit-8 assays. **P<0.01 and ***P<0.001. ALOX15, 12/15-lipoxygenase; FGFR2, fibroblast growth factor receptor 2; HCAECs, human primary coronary artery endothelial cells; OE, overexpression; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR; siRNA, small interfering RNA.

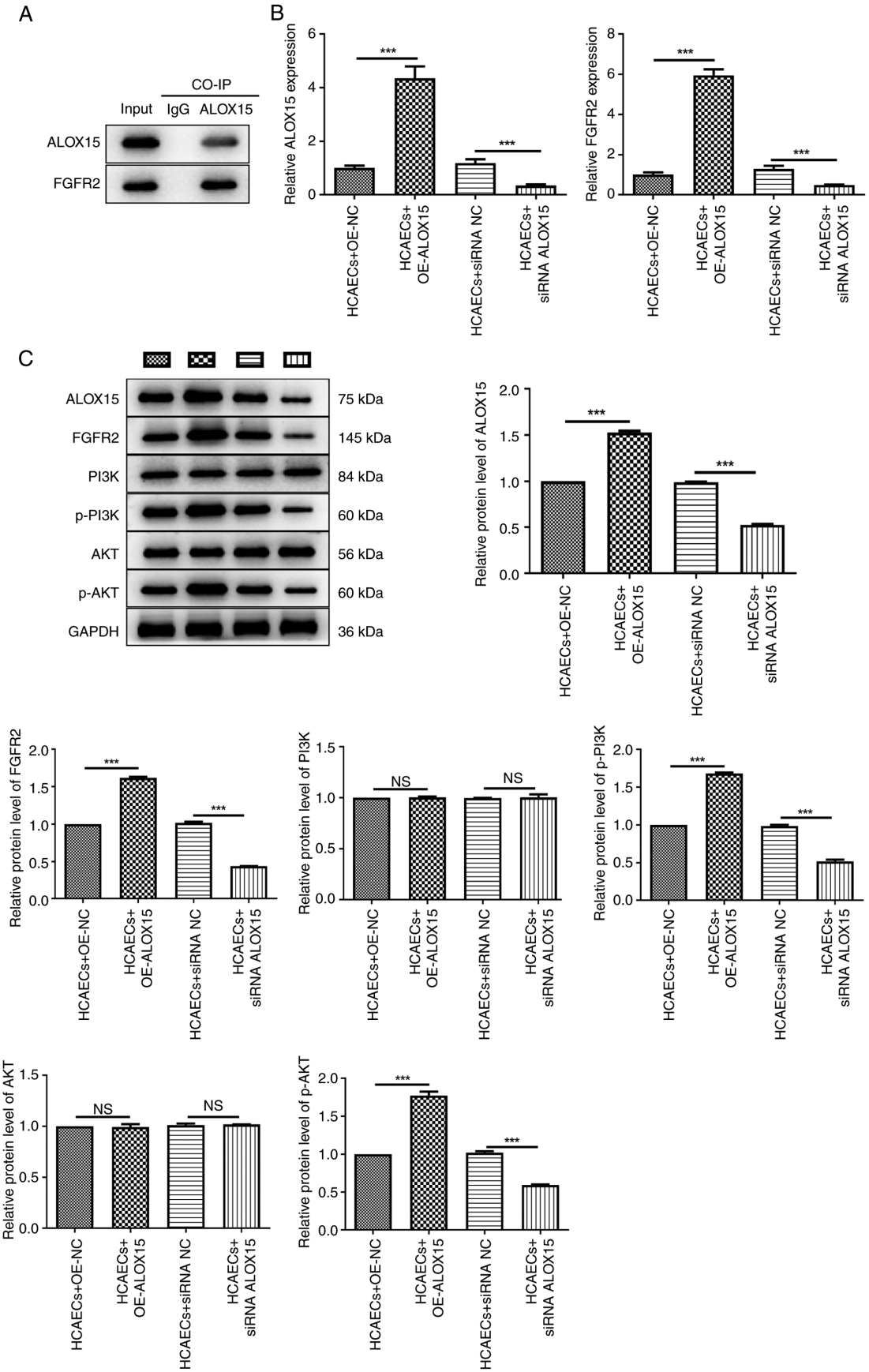

Figure 3. Effects of ALOX15 on the FGFR2/PI3K/AKT signaling pathway in HCAECs. (A) Co-immunoprecipitation assays of ALOX15 and FGFR2 in HCAECs. (B) The mRNA expression of ALOX15 and FGFR2 in HCAECs after transfection with OE-ALOX15/OE-NC or siRNA ALOX15/siRNA NC was detected by reverse transcription-quantitative PCR. ***P<0.001. (C) The protein levels of ALOX15, FGFR2, PI3K, p-PI3K, AKT and p-AKT in HCAECs after transfection with OE-ALOX15/OE-NC or siRNA ALOX15/siRNA NC were determined by western blotting. ***P<0.001. ALOX15, 12/15-lipoxygenase; FGFR2, fibroblast growth factor receptor 2; HCAECs, human primary coronary artery endothelial cells; OE, overexpression; NC, negative control; siRNA, small interfering RNA; p-, phosphorylated; NS, not significant.

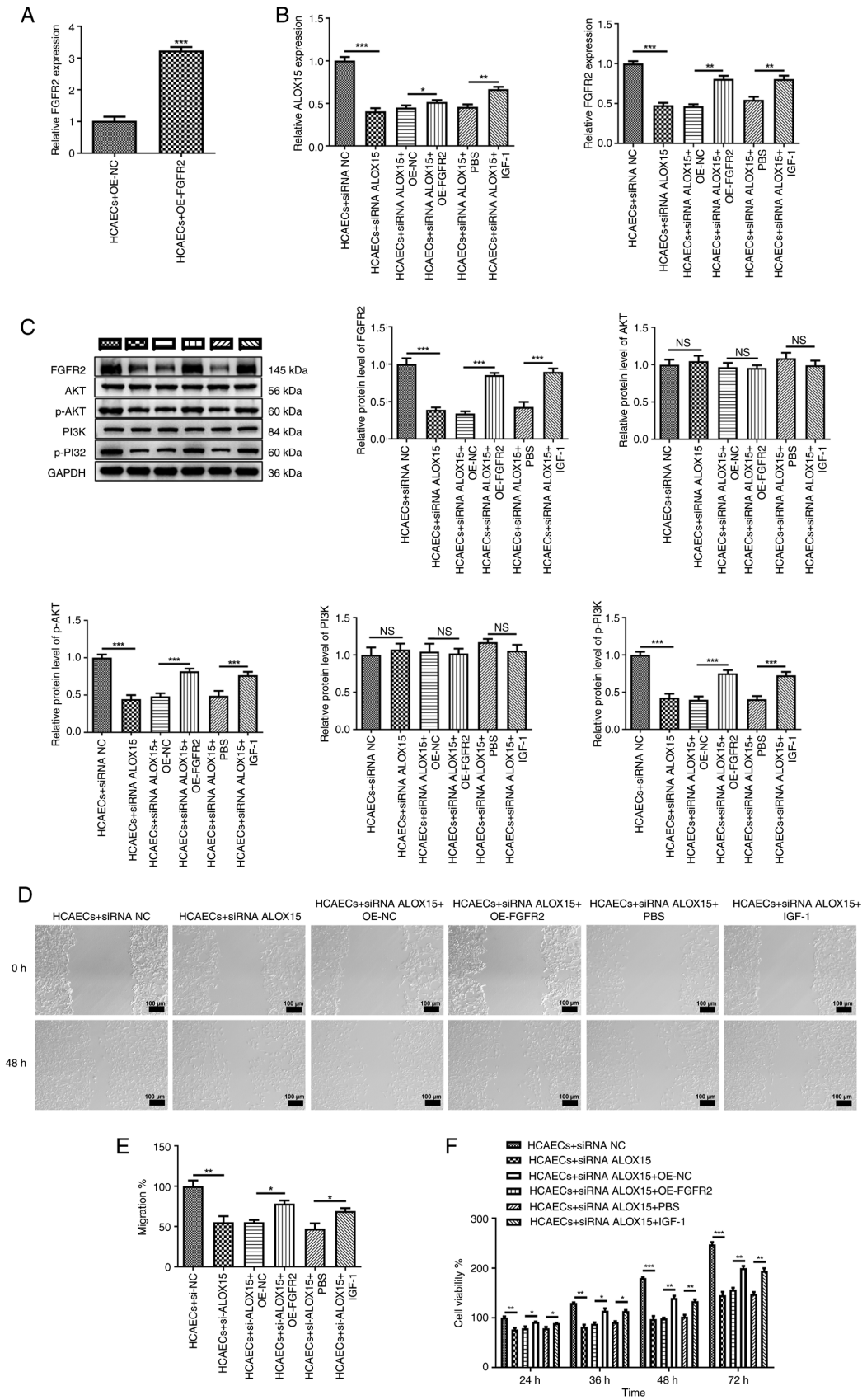

Figure 4. Silencing of ALOX15 mitigates acute coronary syndrome progression *in vitro* through the FGFR2/PI3K/AKT signaling pathway. (A) The expression of FGFR2 in HCAECs after transfection with OE-FGFR2 or OE-NC was detected by RT-qPCR. (B) The mRNA expression of ALOX15 and FGFR2 in HCAECs following different treatments was detected by RT-qPCR. (C) The protein levels of FGFR2, PI3K, p-PI3K, AKT and p-AKT in HCAECs following various treatments were determined by western blotting. (D, E) The migration of HCAECs following different treatments was assessed by scratch wound healing assays. Scale bar, 100 μm. (F) The viability of HCAECs following various treatments was measured by Cell Counting Kit-8 assays. *P<0.05, **P<0.01 and ***P<0.001. ALOX15, 12/15-lipoxygenase; FGFR2, fibroblast growth factor receptor 2; HCAECs, human primary coronary artery endothelial cells; OE, overexpression; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR; siRNA, small interfering RNA; p-, phosphorylated; NS, not significant.

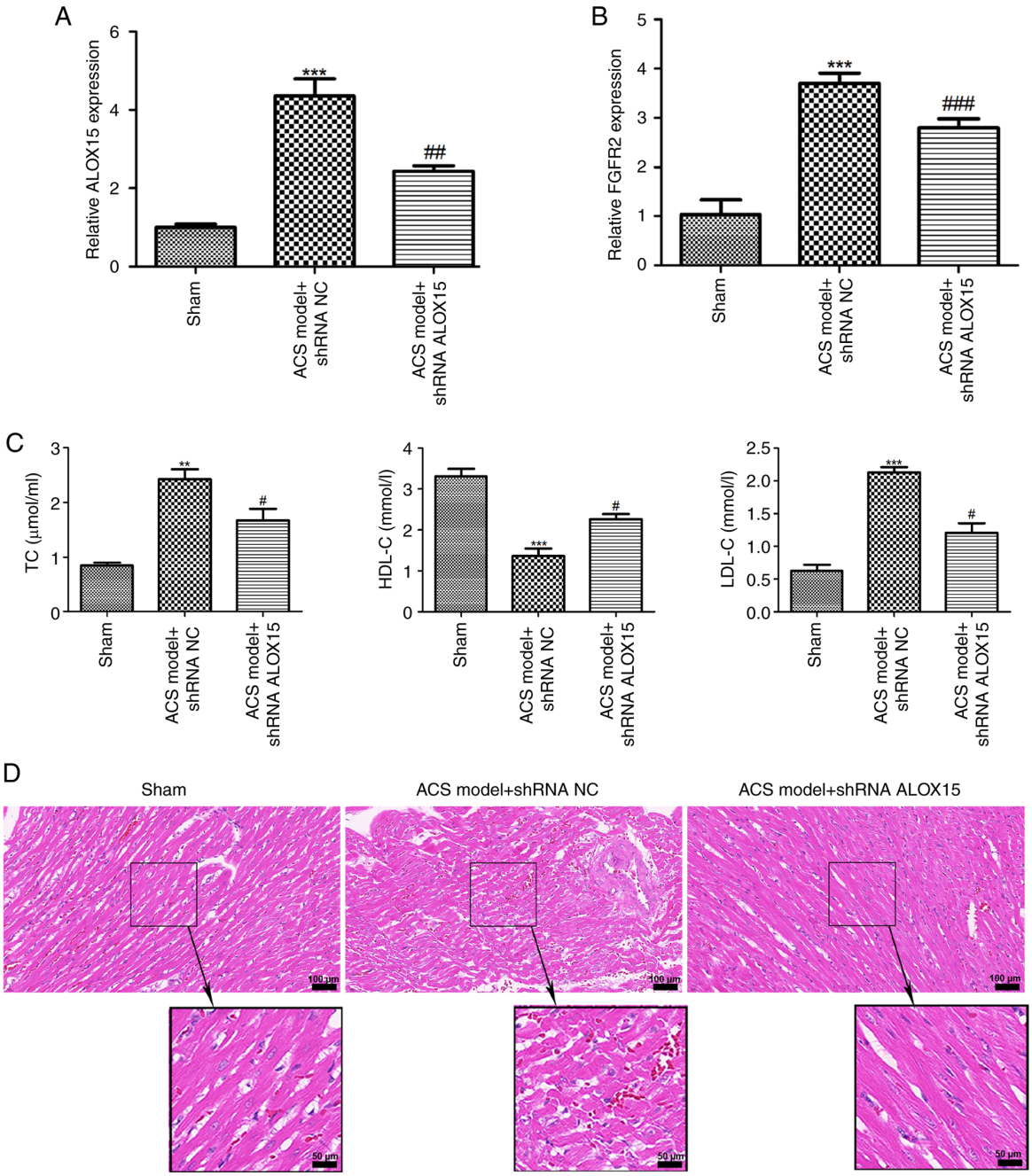

Figure 5. Knockdown of ALOX15 reduces serum lipid levels and improves myocardial injury in an ACS rat model. The expression of (A) ALOX15 and (B) FGFR2 in rats with ACS after injection of shRNA ALOX15 or shRNA NC was detected by reverse transcription-quantitative PCR. ***P<0.001 vs. sham; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. ACS model + shRNA NC. (C) The levels of TC, HDL-C and LDL-C in rats with ACS after injection of shRNA ALOX15 or shRNA NC were determined using biochemical tests. **P<0.01 and ***P<0.001 vs. sham; $^{\#}$P<0.05 vs. ACS model + shRNA NC. (D) Hematoxylin and eosin staining for observing the pathological condition of myocardial tissues in different groups. Scale bars, 100 and 50 μm. ALOX15, 12/15-lipoxygenase; ACS, acute coronary syndrome; FGFR2, fibroblast growth factor receptor 2; shRNA, short hairpin RNA; NC, negative control; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

from the sham group exhibited a normal morphology, with a neat arrangement and no breaks. Conversely, cardiomyocytes from the rats with ACS became swollen and thickened, with irregular morphology and disordered arrangement. Following intramyocardial injection of shRNA ALOX15, a marked improvement in both the morphology and arrangement of cardiomyocytes was noted.

ALOX15 silencing suppresses the activation of the FGFR2/PI3K/AKT signaling pathway. The levels of FGFR2/PI3K/AKT pathway-related proteins were assessed in rats with ACS, as presented in Fig. 6. Western blot analysis revealed a pronounced upregulation of ALOX15, FGFR2, p-PI3K and p-AKT proteins in rats with ACS compared with these levels in the sham rats (P<0.001). Notably, intramyocardial injection of shRNA ALOX15 effectively inhibited the increase of these proteins induced by the ACS challenge (P<0.001). Furthermore, it was determined that both ACS challenge and shRNA ALOX15 treatment did not significantly affect the protein levels of PI3K and AKT.

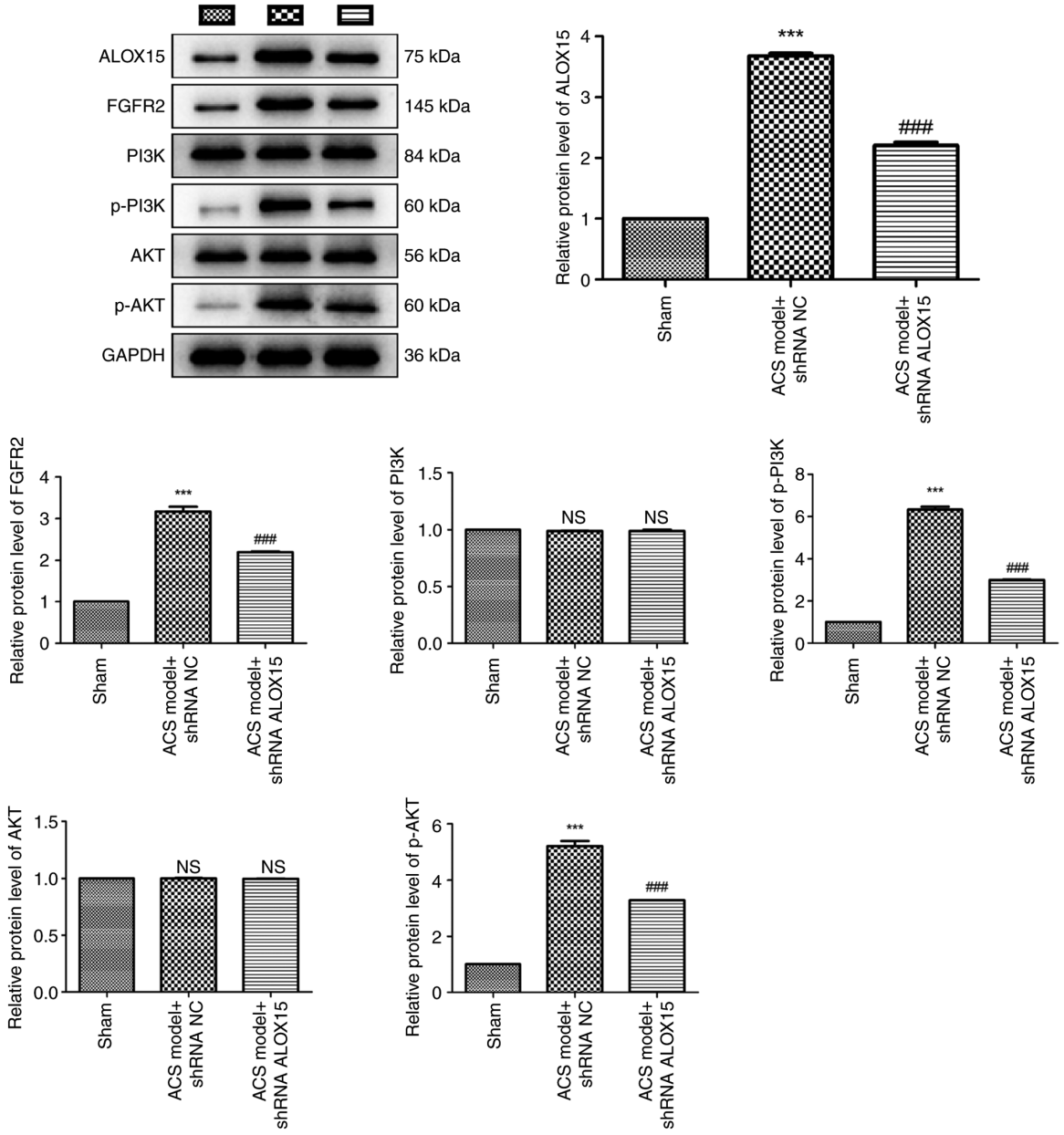

Figure 6. ALOX15 silencing suppresses the activation of the FGFR2/PI3K/AKT signaling pathway. The protein levels of ALOX15, FGFR2, PI3K, p-PI3K, AKT and p-AKT in rats with ACS after injection of shRNA ALOX15 or shRNA NC were determined by western blotting. ***P<0.001 vs. sham; $^{###}$P<0.001 vs. ACS model + shRNA NC. ALOX15, 12/15-lipoxygenase; FGFR2, fibroblast growth factor receptor 2; p-, phosphorylated; ACS, acute coronary syndrome; shRNA, short hairpin RNA; NC, negative control; NS, no significance.

Discussion

Although the mortality of CAD among older patients has recently decreased, the decrease in younger patients, particularly in young women, has been less pronounced (35,36). In spite of this, this condition continues to account for approximately one-third of all deaths in individuals over the age of 35 (37-39). Additionally, the annual cost for each patient with ACS is substantial and staggering, with hospitalization expenses alone nearing $35,000 USD (40). In the USA, the direct costs that the government spends on patients with ACS every year amount to at least $310 billion (41). Consequently, ACS has become a heavy burden not only for patients alone but also for the whole society. In the present study, a novel molecular target for the potential therapy of ACS was expounded, indicating that knockdown of ALOX15 may improve ACS by inhibiting the FGFR2/PI3K/AKT signaling pathway.

The pathology of ACS encompasses multiple aspects, with plaque rupture identified as one of the primary etiological factors (3,4). The proliferation and migration of endothelial cells following myocardial infarction are critical for angiogenesis; however, abnormal endothelial cell behavior may contribute to plaque instability and erosion, ultimately precipitating the onset and progression of ACS (42-44). In the present study, it was demonstrated that the proliferative and migratory abilities of HCAECs were overtly weakened following siRNA ALOX15 transfection, but enhanced by ALOX15 overexpression. These findings indicated that the expression levels of ALOX15 may be associated with ACS progression. Furthermore, endothelial dysfunction is also characterized

by elevated levels of TC and LDL-C (45,46). Since it was confirmed that silencing of ALOX15 inhibited the aberrant proliferation and migration of HCAECs, it was further hypothesized that ALOX15 silencing may also play a significant role in regulating blood lipid levels. As anticipated, the findings in the present study confirmed that injection of shRNA ALOX15 markedly reduced the levels of TC and LDL-C, and elevated the levels of HDL-C in rats with ACS. By determining blood lipid levels in patients with ACS, it can be assessed whether ALOX15 is involved in ACS progression, thereby enabling more ACS targeted clinical treatments. Evidence has indicated that high levels of ALOX15 are correlated with endothelial cell barrier dysfunction in rats with a high-fat diet (47). The results of the present study indicated that knockdown of ALOX15 can relieve endothelial dysfunction, to some extent. In addition, silencing of ALOX15 also alleviated the cardiac injury of rats with ACS. Therefore, it is proposed that ALOX15 may serve as a potential therapeutic target for ACS in clinical settings. Furthermore, the silencing of ALOX15 appears to be beneficial in mitigating the progression of ACS.

It is well-known that FGFR2 is strongly associated with endothelial dysfunction, including aberrant cell proliferation and migration (26,28). Given the significant effects of ALOX15 on the viability and migration of HCAECs, it was hypothesized that ALOX15 may interact with FGFR2 in ACS progression *in vitro*. In the present study, CO-IP confirmed that ALOX15 protein interacts with FGFR2 in HCAECs. Furthermore, it was demonstrated that ALOX15 overexpression increases both the mRNA expression and protein levels of FGFR2, while ALOX15 silencing leads to their reduction. These results indicated that ALOX15 may synergistically interact with FGFR2 to affect the development of ACS. Notably, FGFR2 has been widely reported as an activator of the PI3K/AKT pathway in various human diseases (30,31). Additionally, the PI3K/AKT pathway has been demonstrated to be involved in regulating the growth and function of cardiomyocytes, thrombogenesis and vascular homeostasis (48). Previous studies have reported that PI3K/AKT is a classical signaling pathway involved in numerous cardiovascular disorders such as myocardial ischemia/reperfusion injury, cardiac hypertrophy, myocardial infarction and heart failure (18-21). It was therefore further hypothesized that PI3K/AKT can be modulated by ALOX15 in ACS pathobiology. As anticipated, overexpression of ALOX15 markedly activated the PI3K/AKT pathway. Conversely, silencing of ALOX15 inhibited the PI3K/AKT pathway both in HCAECs and rats with ACS. Notably, both the overexpression of FGFR2 and the addition with IGF-1 significantly mitigated the inhibitory effects of ALOX15 knockdown on the migratory and proliferative abilities of HCAECs. It was therefore concluded that silencing of ALOX15 may suppress the progression of ACS by inhibiting the FGFR2/PI3K/AKT pathway.

Some limitations of the present study should be mentioned. First, in addition to the FGFR2/PI3K/AKT pathway, other potential pathways or mechanisms regulated by ALOX15 in the progression of ACS may exist and should be further investigated in depth. Second, with the exception of the rat model that was established in the present study, similar models have also been extensively used in other animal species, including rabbits (49), mice (50), and pigs (51). In future studies, the findings of the present study will be validated in other animal models. Third, this study was conducted in a specific population in Wenzhou, which may limit the generalizability of the results. In future studies, the research will be expanded to include diverse populations across China. Fourth, elevated levels of ALOX15 may also be influenced by unrelated inflammatory processes. Future studies will better control for such potential confounders.

Additionally, several potential challenges or limitations may arise when translating the current findings from preclinical models to clinical settings. For example, a) physiological differences: i) Targeted drugs can be effectively metabolized in animals, but may be metabolized slowly or produce different metabolites in humans, resulting in differences in efficacy and toxicity; ii) animal models are difficult to fully simulate the complexity of human diseases. b) Drug response differences: i) Targeted drugs that have shown promising efficacy in preclinical models may have reduced efficacy in clinical settings due to various factors; ii) animals often differ in their tolerance and response to drug toxicity compared with humans. Some drugs that may not show significant toxicity in animal experiments, may cause serious adverse reactions in humans. c) External environmental differences: i) Preclinical animals are typically housed in controlled environments with consistent temperature, humidity, light cycles, and diets, however, human lifestyles and environments are highly variable, which can affect treatment outcomes; ii) preclinical models cannot simulate the psychological and social factors of human patients. The psychological state of human patients, such as stress, anxiety, depression, and other emotions, can have an impact on the development and treatment response of the disease.

In summary, the present study provides preliminary insights into the underlying mechanism of ALOX15 in the pathogenesis of ACS. The findings, for the first time, to the best of the authors' knowledge indicate that silencing of ALOX15 may mitigate ACS progression by inhibiting the FGFR2/PI3K/AKT pathway. This research offers a foundational basis and perspectives on potential clinical therapeutic strategies for ACS. However, further investigations to validate these findings in clinical settings are urgently warranted.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LY made substantial contributions to the conception and design of the study. HC, NZ, SH, FG, SH and FY made substantial

contributions to the acquisition, analysis and interpretation of the data. HC drafted the manuscript. NZ, SH, FG, SH and FY revised the manuscript critically for important intellectual content. All authors confirm the authenticity of all the raw data, as well as read and approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The patients/participants provided their written informed consent to participate in the present study. The study was conducted according to the guidelines of the Declaration of Helsinki and approved (approval no. 2020-351) by the Ethics Committee of The Third Affiliated Hospital of Shanghai University (Wenzhou People's Hospital; Wenzhou, China). Animal experiments were conducted in compliance with the Guidelines for the Use of Laboratory Animals and approved (approval no. xmsq2023-1367) by the Ethics Committee of Wenzhou Medical University (Wenzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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