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RESEARCH ARTICLE

miR-135a-5p Regulates Inflammation-Induced Myocardial Fibrosis by Targeting APCs (Adenomatous Polyposis Coli)

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ABSTRACT

Myocardial fibrosis is a critical pathological process contributing to heart failure, characterized by excessive extracellular matrix (ECM) deposition and inflammation. This study investigates the role of miR-135a-5p and its target gene APC in MF pathogenesis. Bioinformatics analysis using TargetScan and the Human miRNA Tissue Atlas revealed tissue-specific expression of miR-135a-5p, with significant levels in the myocardium, thyroid, and epididymis. An *in vivo* rat MF model was established using cardiac ischemia/reperfusion injury, confirmed by histopathological changes, including collagen deposition and loss of myocyte nuclei. miR-135a-5p was upregulated in MF tissues, and its modulation significantly influenced fibrosis and inflammation. miR-135a-5p mimics increased fibrosis markers (α-SMA, Col1a1, Collagen II) and pro-inflammatory cytokines (TNF-α, IL-6), while miR-135a-5p inhibition attenuated these effects. TargetScan and dual luciferase assays identified APC as a direct target of miR-135a-5p, with miR-135a-5p binding to the 3'UTR of APC mRNA. The effect of APC on fibrosis markers and inflammatory factors in the rat MF model was detected. Co-activation of miR-135a-5p and APC in the rat MF model attenuated fibrosis and inflammation, highlighting the regulatory role of the miR-135a-5p/APC axis. *In vitro*, Ang II-stimulated human atrial fibroblasts (HAFs) exhibited elevated miR-135a-5p, reduced APC, and increased fibrosis markers and proliferation. miR-135a-5p activation promoted fibrosis and inflammation, while activation of APC attenuates miR-135a-5p-induced fibrosis and inflammation in myocardial tissues. These findings highlight the miR-135a-5p/APC axis as a potential therapeutic target for MF.

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INTRODUCTION

Myocardial fibrosis (MF) is a critical contributor to the development of heart failure, as it induces cardiovascular stiffness and subsequent damage (Manka *et al.*, 2019). Consequently, MF has emerged as a key diagnostic marker for heart disease in animals (Talman *et al.*, 2016; Gan *et al.*, 2018; Song *et al.*, 2020), and dysregulated collagen turnover and imbalances in pro- and anti-fibrotic factors (Li *et al.*, 2014; Heymans *et al.*, 2015). However, the molecular mechanisms driving MF remain poorly understood, and a deeper understanding of these processes could facilitate the development of targeted therapies.

Three hallmark features characterize MF development: a rapid proliferation of cardiac fibroblasts, fibroblast-to-myofibroblast transition (FMT), and excessive extracellular matrix (ECM) deposition. During cardiovascular injury, angiotensin II (Ang II) activates transforming growth factor-β (TGF-β) signaling, which mediates MF through fibroblast accumulation and FMT (Ghosh *et al.*, 2013).Fibrogenesis and subsequent cardiac remodeling are regulated by diverse pathophysiological triggers, including inflammatory responses, oxidative stress, and cellular senescence (Kong *et al.*, 2014). The ratio of sudden cardiac death (SCD) in young athletic horses was estimated to be approximately 124-158

deaths/100,000 horses per year. But the ratio increased more, with an estimated ratio of 1/15,000 to 1/100,000 human athlete years per year (Physick-Sheard *et al*., 2019; Morrice-West *et al*., 2020). The occurrence of MF enhances inflammatory processes, leading to elevated levels of pro-inflammatory factors in patients, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and growth differentiation factor 15 (GDF15) (Rullman *et al.,* 2020). TNF-α and IL-6 activate inflammation-associated cells, triggering a cascade of cellular inflammation and matrix metalloproteinase activation (Nian *et al.,* 2004; Paulus *et al.,* 2021). Activated cardiac fibroblasts undergo phenotypic changes, differentiating into myofibroblasts characterized by ECM protein production and α-smooth muscle actin (αSMA) expression (Pandya *et al.,* 2006; Gandhi *et al.,* 2011; Pchejetski *et al.,* 2012; Shinde *et al.,* 2014). Thus, reducing myocardial inflammation can mitigate MF by inhibiting fibroblast activation and ECM accumulation.

MicroRNAs (miRNAs), short non-coding RNAs, selectively bind to the 3′-UTR of target mRNAs. miRNAs also are pivotal in regulating protein-coding genes involved in fibrosis and inflammation (Piccoli *et al.,* 2015). Specific miRNAs influence fibrotic pathways in MF development. For instance, miR-451a and miR-214-3p exhibit anti-fibrotic properties, while miR-99-3p and miR-21 promote fibrosis (Gupta *et al.,* 2016; Yang *et al.,* 2019; Deng *et al.,* 2022). miR-125b enhances fibrosis by regulating p53 (Nagpal *et al.,* 2016), whereas miR-29b inhibits TGF-β signaling, offering protection against MF (Zhang *et al.,* 2014). These findings highlight the therapeutic potential of miRNAs in attenuating fibrotic damage. Previous literature address and shown that the hsa-miR-135a-5p may serve as a potential biomarker for myocardial fibrosis following acute myocardial infarction (AMI), as its levels are elevated in post-MI fibrosis (Wang *et al.,* 2021). Additionally, miR-135a-5p is upregulated in cardiac hypoxic/ischemic injuries and mitigates hypoxia-induced damage in H9c2 cardiomyocytes (Zhi *et al., 2016; Xu et al.,* 2020). miR-135a-5p was selected due to its elevated expression in post-MI fibrosis, and APC was identified as a potential target through bioinformatics analysis using TargetScan. Previous studies have shown that miR-135a-5p is upregulated in cardiac hypoxic/ischemic injuries, suggesting its role in fibrosis and inflammation. These studies underscore the significant role of miR-135a-5p in MF.

The mechanisms underlying MF in animals mirror those in humans, including oxidative stress, inflammation, and dysregulated extracellular matrix (ECM) deposition (Martins *et al*., 2021). Therefore, understanding the molecular pathways driving MF is critical for developing targeted therapies to improve cardiac health in patients (Reis-Ferreira *et al*., 2022). Understanding the role of miRNAs like miR-135a-5p in MF could provide insights into novel therapeutic strategies for managing cardiac fibrosis in both human and animal (Wen *et al*., 2021). Based on these findings, this study aimed to elucidate the precise role of miR-135a-5p in MF in rat's model and its interaction with APC also underlying the molecular mechanisms.

MATERIALS AND METHODS

Detection of miR-135a-5p abundance in different tissues: The gene search was conducted using miRTarBase (Version 4.5), employing the search term 'miR-135a-5p' to identify target genes. miR-135a-5p expression was analyzed in the myocardium, thyroid, and epididymis using the"Human miRNA Tissue Atlas" option, and the analysis graph was downloaded online.

Gene interaction analysis: Candidate Differently Expressed Genes (DEGs) were added to the online database Human MicroRNA Disease Database (HMDD v3.2). The "miR-Target Network" was used to find positive and negative regulatory genes of miR-135a, and the interacting molecular divergence maps were downloaded online.

Establishment of the rats MF model: This research was approved by the Animal Ethics Committee of The First Affiliated Hospital of Qiqihar Medical University 2022 (No.16). Sprague-Dawley (SD) male rats aged 8-10 weeks were purchased from Cyagen Biosciences. The rats were kept in animal chambers with SPF conditions, following a light/dark cycle of 12 hours each. The *In-vivo* rat MF model was constructed by inducing cardiac ischemia/reperfusion injury (Xu *et al*., 2020).. Plasmids of different components (mimics-NC, siRNA-NC, mimics-miR-135a-5p, OE-NC, siRNA-miR-135a-5p, OE-APC, mimics-miR-135a-5p+OE-APC, sh-NC, sh-APC) were injected at 20nmol immediately after modeling and sampled one week later. The administered components were diluted in phosphate-buffered saline (PBS). The experimental groups were as follows: Sham group, MF group, mimics-miR-135a-5p, mimics-NC group, siRNA-NC, sh-NC, OE-NC, siRNA-miR-135a-5p, OE-APC, sh-APC, mimics-miR-135a-5p+OE-APC. There were 6 rats in each group (n=6). At the end of the *in vivo* study, rats were anesthetized using ketamine/xylazine followed by euthanasia, and samples (myocardium, thyroid, and epididymis) were collected for further analysis.

Cells Culture: Human atrial fibroblast (HAFs) cells were initially cultured in serum-free medium 24 hours to synchronize the cells before experiments. After 24 hours HAFs Cell (HAFs, Cell Research Co., Ltd. Shanghai, China) were cultured in DMEM medium containing Neuro Cult™ Proliferation Supplement (Stem Cell Technologies), 1% N2 (Gibco), 20ng/mL basic fibroblast growth factor (BGF; R&D Systems), 20ng/mL epidermal growth factor (EGF), and 1% penicillin-streptomycin (Sigma) in 95% air and 5% CO_2 at 37°C and authenticated by STR profiling .

Establishment and identification of the MF cell model: The MF cell model was established by stimulating HAFs with 1μM Ang II for 24 hours. Cells were allowed to adhere and adjust for 24 hours before adding the MTT solution. Ang II (1μM) was added in the experimental group, while no Ang II was in the control group. The cells of the two groups were cultured

for 24h and then observed to identify the cell proliferation viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells of the two groups ($1x10^4$ cells/well) were cultured at 37°C for 3 hours with 20μL of MTT solution. After 3 hours of incubation with MTT, DMSO (150μL, Sigma) was added to dissolve the formazan crystals. After 15 minutes, absorbance was measured by using a microplate reader (BioTek, USA) at 570nm, and untreated wells were used as controls.

Cell transfection: HAFs cells ($2×10^5$ cells/well) were inoculated into 6-well plates. miR-135a-5p inhibitor (100nM;5'-AUAACCGAAAAAAAUAAGAUACACU-3'), miR-NC mimic (50nM; 5'-CUGAACUGCUGGACGCGUA-3'), miRNA negative control (NC) inhibitor (100nM; 5'-CUGAUAGCGCAUGUCCGAGCUA-3'), miR-135a-5p mimic (50nM; 3'-AUGUAUUCCUUUUCGGUAU-3'), si-NC and siRNA- APC (50nM) were purchased from Sigma (USA). HAF cells were subjected to transfection using Lipofectamine 2000 (Invitrogen). Following an incubation period of 48 hours at a temperature of 37C, the cells were harvested for further experimentation.

RT-qPCR: The TRIzol reagent (Invitrogen) was employed to extract total RNA from HAFs cells. The PrimeScript RT kit (Takara) was used for reverse transcription of the extracted total RNA into cDNA. Total RNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher, USA), and RNA purity was assessed using the A260/A280 ratio. The RT-qPCR was conducted utilizing the ABI Prism 7500 instrument from Applied Biosystems, along with SYBR-Green mix kits provided by Roche Diagnostics. The $2^{-\Delta\Delta}$CT method was employed to quantify the relative expression of miR-135a-5p as well as APC, with U6 and β-actin serving as internal reference genes. The primer sequences can be found in Table 1.

Table I: Primer sequences used for RT-qPCR

Gene	Forward primer	Reverse primer
U6	5'CGCCAAGGATGACACGCAAAT-3'	5'-GCGGTCCGAGGTATTC-3'
miR-135a-5	5'-AGTUATCTTTTCGGTAT-3'	5'ATACGAAAAATAAGGATACACT-3'
β-actin	5'-ATGTGCGACGAACGAGAC-3'	5'-CCTTCGACCCATACCACCAT-3'
APC	5'GCCCTTTGTAACTCCGATGG-3'	5'GGAGGATGGTGACGTTTTGG-3'

ELISA: Blood samples were collected via cardiac puncture and tissue samples by euthanizing the rats and were centrifuged at 3000 rpm for 10 minutes to obtain serum. Serum samples of each group of rats were collected one week after model establishment, and supernatants from each group of cells were separated, and TNF-α (KRC3011C) as well as IL-6 (88-7064-88) in rat serum and cell supernatant were indicated using ELISA kits (eBioscience).

Dual luciferase reporter assay: The dual luciferase reporter assay was employed to evaluate the interplay between APC and miR-135a-5p. The sequences of the wild-type (WT) APC mRNA 3' untranslated region (UTR) or mutant (Mut) APC mRNA 3' UTR were subcloned into the pmirGLO plasmid (Promega, USA) by Thermo Fisher. The miR-135a-5p mimic/NC and pmirGLO WT APC 3'UTR/pmirGLO-Mut APC3'UTR were co-transfected into HEK293T cells. Two days later, fluorokinase activity was quantified by a dual fluorokinase reporter assay system (Promega, USA).

HE staining: Myocardial tissues were treated using 4% paraformaldehyde, and tissues were dehydrated in 70% ethanol. Then tissues were embedded in paraffin and cut into 5-8μM thick sections and stained using a hematoxylin-eosin staining kit (Solarbio Technology).

CCK-8: The cells were incubated at 37°C with a CO_2 concentration of 5% in the culture medium. Then, a serum-free medium was introduced, and Lipofectamine 3000 was utilized for transfecting each group of transfection plasmids into the cells. The serum-free medium was replaced with the proliferation medium after 4 hours of transfection. Afterward, a volume of 10μL CCK-8 solution was introduced and subjected to incubation in the designated apparatus for 1-4 hours. $1×10^4$ cells were plated per well and allowed to adhere for 24 hours before transfection. Subsequently, the OD measurements were taken at 24, 48, and 72 hours by using a wavelength of 450nm.

Statistical analysis: Each experiment was performed three times. All statistical analyses were conducted utilizing GraphPad Prism 7.0 software. The outcomes are presented as the mean value accompanied by the standard deviation. Statistical comparisons were conducted between the two groups utilizing t-tests. Statistical analyses of three or more groups were analyzed using one-way ANOVA and the Mann-Whitney U test or Tukey's post hoc test. The statistical significance was determined by considering P-values below 0.05 ($P<0.05$).

RESULTS

miR-135a-5p expression across various tissues: Analysis of the "Human miRNA Tissue Atlas" through bioinformatics reveals that miR-135a-5p is differentially expressed across various tissues, with significantly ($P<0.05$) higher abundance in tissues such as thyroid and epididymis compared to other tissues. Additionally, miR-135a-5p was found to be expressed in control myocardium as well (Fig. 1A).

Establishment of the Rat Myocardial Fibrosis (MF) Model: miR-135a-5p expression was analyzed in various tissues, including the myocardium, thyroid, and epididymis, by using bioinformatics. The results indicated that cardiac perfusion injury induces significant myocardial fibrosis in rats. H&E staining revealed structural disorganization and loss of myocyte nuclei in the infarcted area. HE staining revealed a significant ($P<0.05$) increase in collagen deposition in the myocardium of MF rats compared to sham controls. Additionally, Histological changes, including loss of transverse striations and unclear myofibrillar structure, were observed in the infarcted area. (Fig. 1B). Additionally, miR-135a-5p levels were significantly elevated in MF tissues compared to healthy tissues (Fig. 1C).

miR-135a-5p regulation of myocardial inflammation and fibrosis in rats: The findings revealed that miR-135a-5p mimics upregulated the expression of fibrosis markers α-SMA, Col1a1, and Collagen II, whereas interference with miR-135a-5p decreased these markers (Figure 1D). The results of RT-qPCR indicated that the modulation of miR-135a-5p affected the myocardial inflammation in the fibrotic rats. miR-135a -5p mimics stimulated TNF-α as well as IL-6, whereas interference with miR-135a-5p decreased the expression of TNF-α as well as IL-6 (Fig. 1E).

APC as a target of miR-135a-5p: The potential genes that miR-135a-5p may target were discovered through the utilization of TargetScan (www.TargetScan.org/vert_71). TargetScan analysis identified APC as a potential target of miR-135a-5p, with a binding site in the 3'UTR region of APC mRNA (Figure 2A, B). miR-135a-5p mimics reduced luciferase activity in HAFs cells transfected with wild-type (WT) APC 3'UTR but not in cells with mutant (Mut) APC 3'UTR, confirming direct targeting (Figure 2C). APC expression was significantly lower in MF model rats compared to control myocardium (Fig. 2D).

APC Regulation of Fibrosis and Inflammation in Rats: Molecular experiments revealed that overexpression of APC was responsible for the decreased expression of Collagen II, α-SMA, and Col1a1 in the MF model, whereas inhibition of APC increased the same markers (Figure 2E). Inflammatory responses that similarly mediate the development of fibrosis also showed different degrees of change. APC overexpression reduced TNF-α and IL-6 levels, whereas APC inhibition elevated these cytokines (Fig. 2F).

miR-135a-5p/APC pathway modulates fibrosis and inflammation in rats: Simultaneous regulation of miR-135a-5p, as well as APC in rats MF model, RT-qPCR and ELISA, were performed to detect fibrosis markers and inflammatory factor activities. Activation of miR-135a-5p alone increased fibrosis markers, but co-activation of miR-135a-5p and APC attenuated this effect (Fig. 3A). Additionally, miR-135a-5p activation alone elevated TNF-α and IL-6, but co-activation with APC reduced these inflammatory cytokines (Fig. 3B).

Validation of the MF Cell Model Using Ang II-Stimulated HAFs: The results from the *In vitro* MF cell model using HAFs stimulated by Ang II indicated that Ang II stimulation significantly increased α-SMA, MMPs, and Collagen II levels in HAFs (Figure 4A). Ang II-treated HAFs showed significantly elevated cell viability (Fig. 4B). The findings indicated that 10^{-6} mol/L Ang II successfully induced HAFs proliferation and collagen synthesis, and the MF cell model was successfully constructed. In addition, the study also found that there was a notable miR-135a-5p was upregulated, while APC was downregulated in Ang II-treated cells (Fig. 4C, D).

miR-135a-5p and APC Regulate Fibrosis and Inflammation in HAFs: The results of the *in vitro* study indicated an activation of miR-135a-5p-stimulated α-SMA, MMPs, Collagen II and IL-6, TNF-α while disruption of miR-135a-5p inhibited these factors (Fig. 4E, F). In contrast, activation of APC decreased Collagen II, α-SMA, MMPs, and TNF-α, IL-6, and interference with APC increased these factors (Fig. 4G, H). The findings suggest that miR-135a-5p, as well as APC in HAFs cells, play a regulatory role in Ang II-induced inflammation as well as fibrosis within these cells.

miR-135a-5p/APC pathway modulates inflammation and Fibrosis in MF cell Model: The co-transfection method was employed to achieve the co-activation of miR-135a-5p and APC in Ang II-treated HAFs cells. RT-qPCR indicated the significantly elevated expression of α -SMA, MMPs, and Collagen II in fibrotic cardiac myocytes mediated by miR-135a-5p mimics were attenuated by the APC mimics (Fig. 5A). ELISA revealed that miR-135a-5p mimic-induced fibrotic cardiomyocytes of elevated TNF-α, IL-6 were attenuated by APC mimics (Fig. 5B). The above results suggest that the impact of miR-135a-5p mimics on Ang II-induced fibrotic cardiomyocytes was attenuated by APC mimics. Ultimately, it can be concluded that miR-135a-5p/APC/inflammatory factors can affect fibrosis markers in human cardiomyocyte fibrosis models cultured and induced *in vitro*.

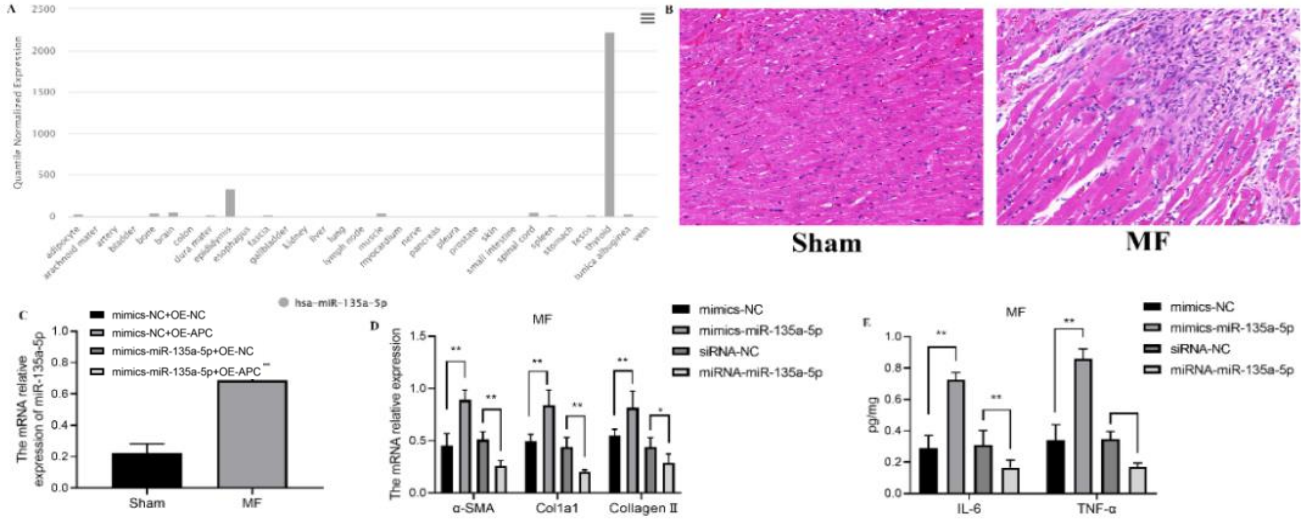

Fig. 1: MiR-135a-5p regulates myocardial inflammation and fibrosis in rats(A). miR-135a-5p in various tissues. (B). HE staining detected the effect of the MF model construction. (C). RT-qPCR showed miR-135a-5p in MF and control group myocardial tissues. (D). The impact of miR-135a-5p on α-SMA, Col1a1, and Collagen II in MF model by RT-qPCR. (E). ELISA revealed the impact of miR-135a-5p on TNF-α as well as IL-6 in the MF model. *P less than 0.05, **P less than 0.01.

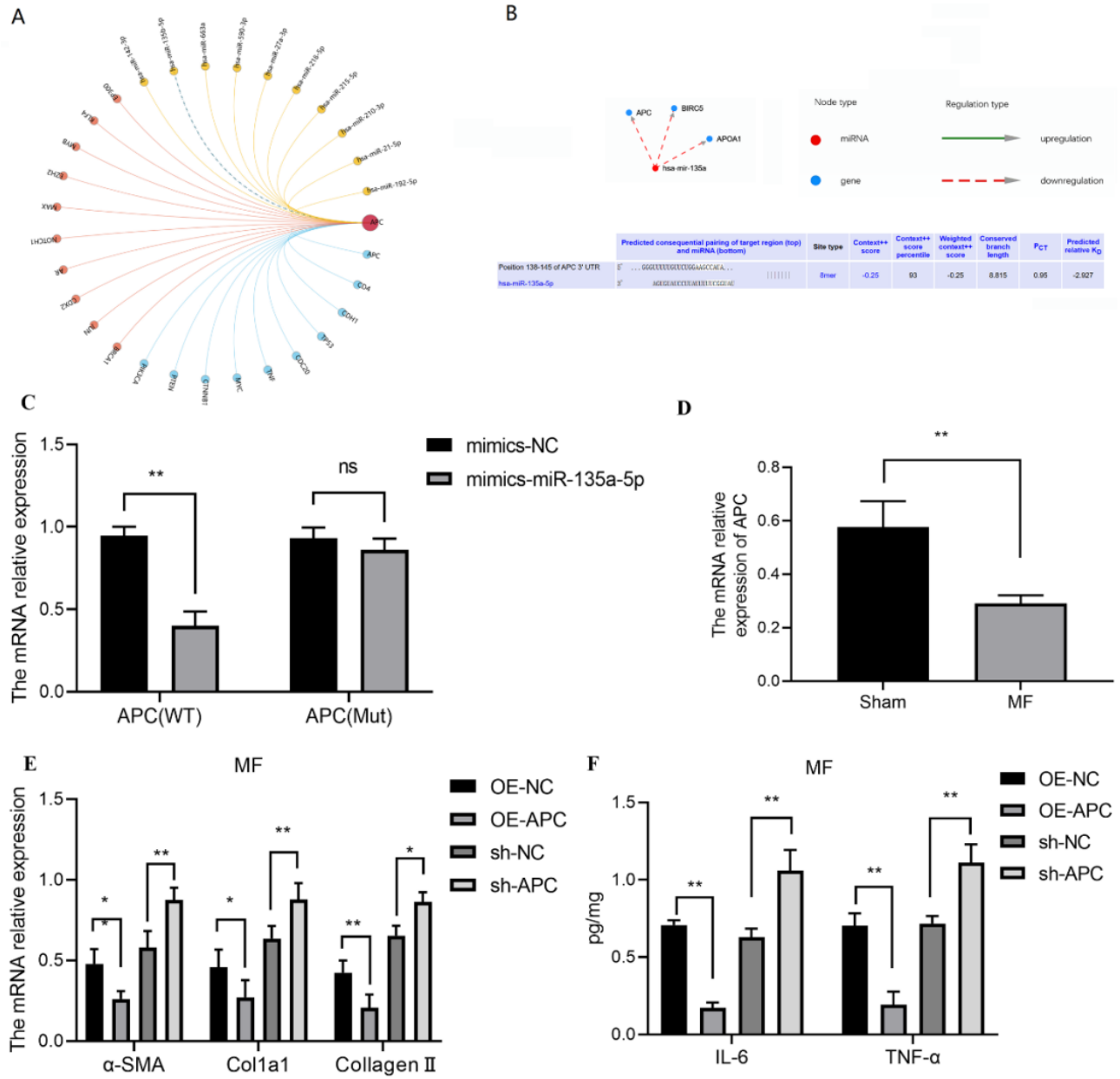

Fig. 2: APC is the target molecule of miR-135a-5p and can regulate myocardial inflammation and fibrosis in rats. (A). Prediction of miR-135a-5p target molecules. (B). Prediction of target sites of miR-135a-5p interaction with APC. (C). RT-qPCR detected miR-135a-5p. (D). APC in the MF model. (E). RT-qPCR showed the effect of APC on α-SMA, Col1a1, and Collagen II in the MF model. (F). ELISA revealed the effect of APC on TNF-α and IL-6 in the MF model. ***P* less than 0.01, **P* less than 0.05, ****P* less than 0.001.

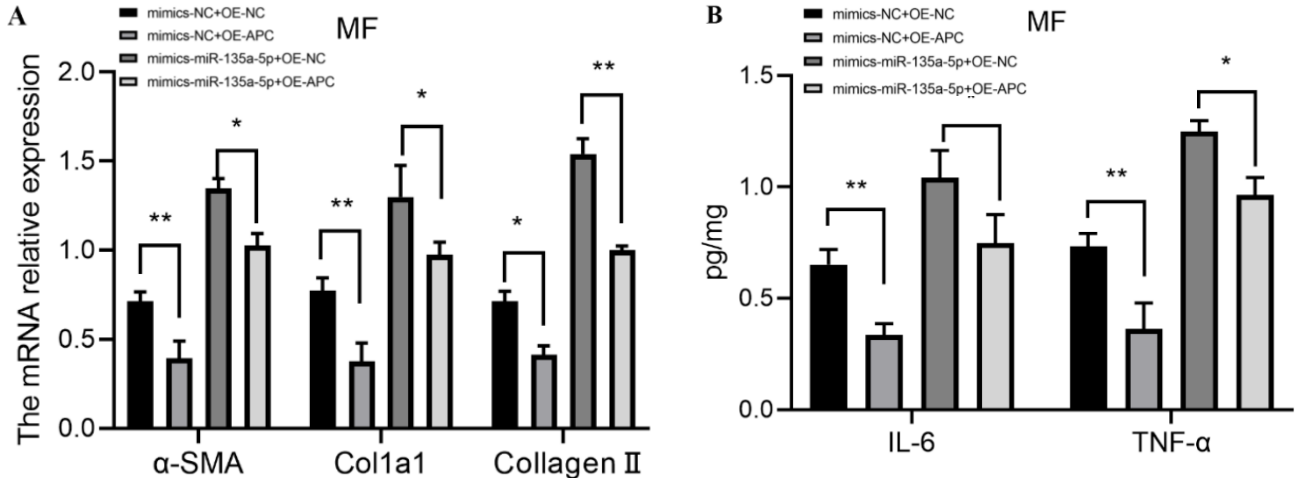

Fig. 3: Regulation of miR-135a-5p/APC pathway affects inflammation and fibrosis in rats MF model (A). Col1a1, α-SMA, and Collagen Ⅱ were determined by RT-qPCR. (B). ELISA examined TNF-α as well as IL-6. **P* less than 0.05, ***P* less than 0.01.

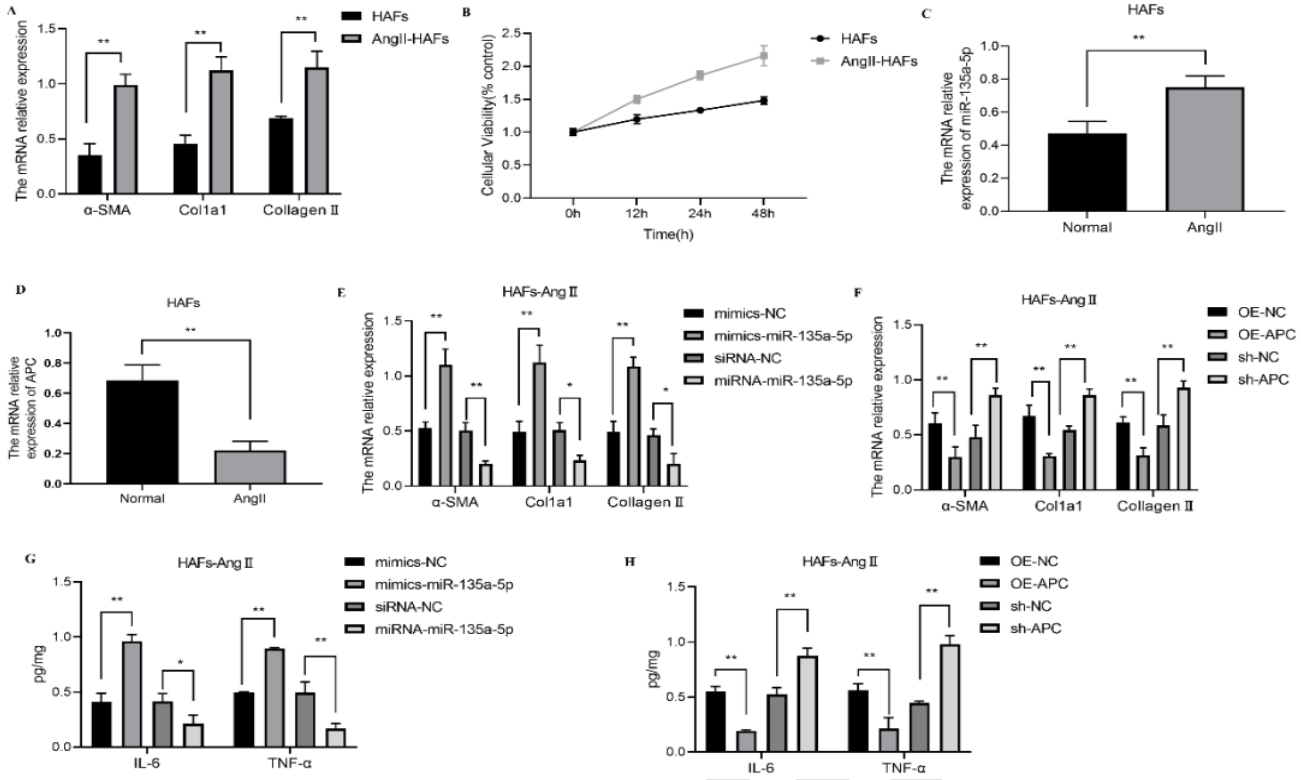

Fig. 4: miR-135a-5p and APC can affect inflammation and fibrosis in the MF model (A). The mRNA of α-SMA, Col1a1, and Collagen II. (B). CCK8 showed the cell viability. (C). The mRNA of miR-135a-5p. (D). The mRNA of APC. (E- F). The mRNA relative expression by RT-qPCR. (G-H). ELISA showed the TNF-α as well as IL-6. ***P* less than 0.01, **P* less than 0.05.

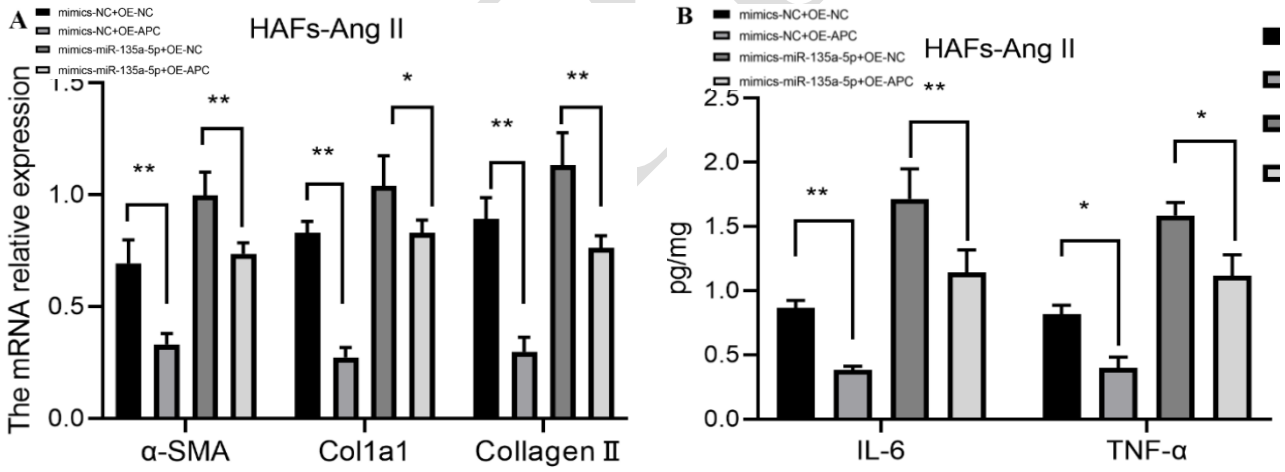

Fig. 5: miR-135a-5p/APC pathway affects inflammation and Fibrosis in MF cell Model, (A), RT-qPCR for the effect of α -SMA, Col1a1 and Collagen Ⅱ expression. (B), ELISA for the effects of TNF- α as well as IL-6. ***P* less than 0.01, **P* less than 0.05.

DISCUSSION

Myocardial fibrosis (MF) is a critical pathological process in cardiovascular diseases, including heart failure, myocardial infarction, hypertension, and diabetes mellitus (Yu *et al.*, 2019). MF drives cardiac remodeling, impairing left ventricular systolic and diastolic function and significantly increasing mortality (Schimmel *et al.*, 2020). Myocardial infarction, often caused by obstruction of coronary blood supply, leads to post-infarction remodeling that depends on the extent of myocardial damage (Spinale *et al.*, 2007; Talman *et al.*, 2016). In this study, our findings demonstrate that miR-135a-5p promotes fibrosis by targeting APC.

MF involves diverse pathophysiological changes, including oxidative stress, collagen deposition, cell death, and inflammation (Kong *et al.*, 2014). Inflammatory responses play a pivotal role in the progression of MF in human and animal studies (Krupkova *et al.*, 2018). Prolonged activation of innate immune pathways exacerbates tissue damage through a robust inflammatory response, which further promotes fibrosis (Mann *et al.*, 2015). Pro-inflammatory cytokines, such as TNF-α and IL-6, are elevated contributing to fibroblast activation and

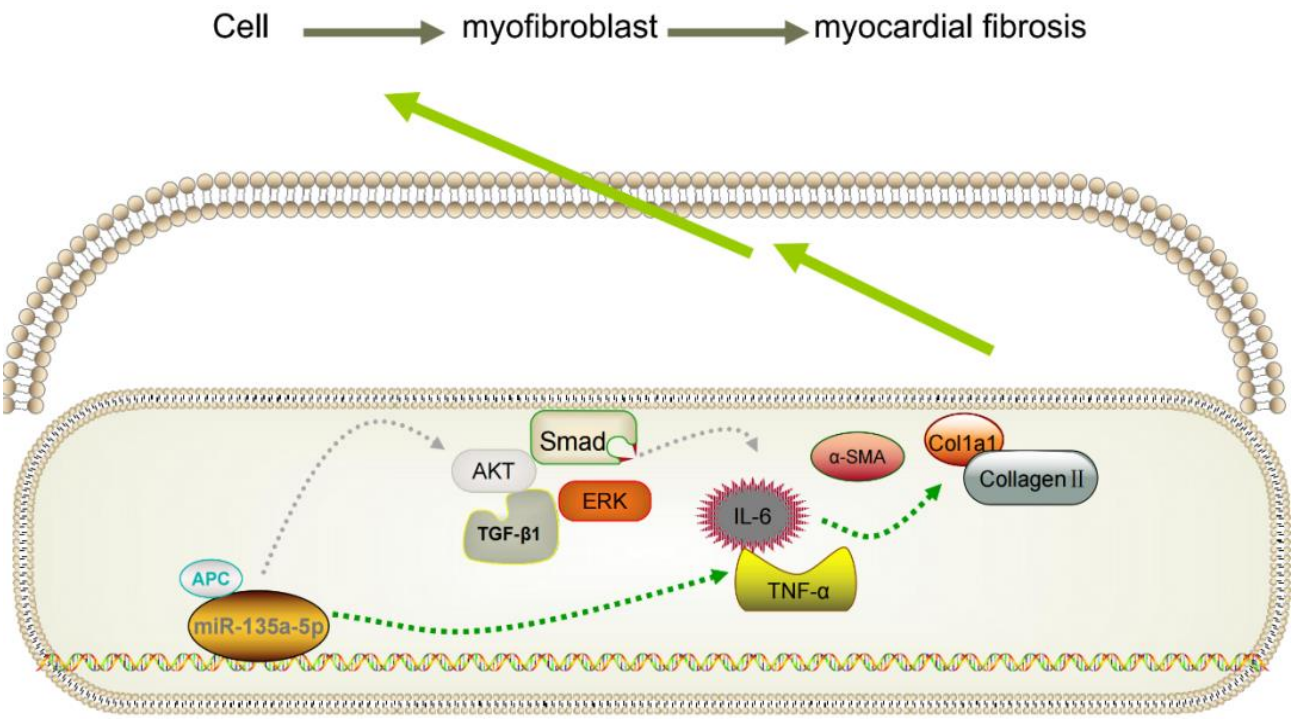

Fig. 6: Schematic diagram of miR-135a-5p / APC pathway affecting inflammation and fibrosis in cardiomyocytes. The interaction of miR-135a-5p with the APC caused an inflammatory response, thereby regulating the fibrosis process.

ECM deposition (Oyama *et al.*, 2008). Chronic inflammation due to endocarditis or myocarditis exacerbates fibrosis, leading to impaired cardiac function (Durando, 2019). The interplay between inflammation and fibrosis in animals underscores the need for therapeutic strategies that target these pathways. The findings of this study, which demonstrate the role of miR-135a-5p in regulating inflammation and fibrosis, could be directly applicable to modern medicine (Ichii *et al.*, 2014; Chen *et al.*, 2024). For instance, targeting miR-135a-5p or its downstream effector APC could mitigate fibrosis and improve cardiac function in animals or human with chronic heart diseases.

Pro-inflammatory cytokines, such as TNF-α and IL-6, stimulate fibroblasts to secrete fibronectin and other mechano-matrix proteins, aggravating ECM deposition and fibrosis (Reddy *et al.*, 2008; Piotti *et al.*, 2024). Thus, controlling pro-inflammatory factor release is essential for mitigating MF.

Cardiac homeostasis is tightly regulated by non-coding RNAs, which play a crucial role in post-transcriptional gene expression in the biological process (Chodkowska *et al.*, 2017; Colpaert *et al.*, 2019; Winter *et al.*, 2022). Most studies evaluated microRNA expression in humans, and despite fewer studies in animals, this topic is one of the most exciting areas of modern medicine. miRNAs showed to be part of the pathogenesis of diseases and reproduction physiology in animals, making them biomarkers candidates (Winter *et al.*, 2022). This study found that miR-135a-5p is highly expressed in rat MF models and Ang II-induced human atrial fibroblasts (HAFs). Silencing miR-135a-5p attenuated myocardial inflammation and fibrosis, consistent with previous findings that miR-135a-5p plays a therapeutic role in isoproterenol-induced MF (Wei *et al.*, 2020). Additionally, miR-135a-5p was shown to target APC, a tumor suppressor gene, and its downregulation exacerbated fibrosis and inflammation. The overexpression of APC inhibited inflammatory factors and attenuated Ang II-induced fibrosis in cardiac fibroblasts, highlighting the significance of the miR-135a-5p/APC axis in MF. These findings suggest that targeting this axis could be a promising therapeutic strategy for MF.

In recent years, miR-135a-5p has been shown to regulate BAG3 in breast cancer, where it inhibits tumor progression. (Vishnuraj, 2020; Diao *et al.*, 2021). This underscores its potential as a diagnostic and therapeutic target across various diseases.

The impact of miR-135a-5p on α-SMA further confirms its pro-fibrotic role (Fig. 6). miR-135a-5p overexpression upregulated α-SMA, Col1a1, and Collagen II, suggesting its involvement in the TGF-β1/Smad/AKT/ERK pathway, which drives fibroblast-to-myofibroblast transition and ECM deposition (Dobaczewski *et al.*, 2011; Giannandrea *et al.*, 2014*l*). Excessive MMPs can lead to ECM remodeling and fibrosis by disrupting the balance between matrix degradation and deposition. These findings elucidate the mechanisms by which miR-135a-5p promotes fibrosis and highlights its potential as a therapeutic target for cardiac patients with MF and cardiomyocyte injury. Further clinical studies are needed to validate these findings and explore the translational potential of targeting the miR-135a-5p/APC axis in MF.

Conclusions: This study demonstrates that miR-135a-5p promotes MF by targeting APCs, while APC activation mitigates fibrosis and inflammation. The miR-135a-5p/APC axis represents a promising therapeutic target for MF, offering new insights into the

molecular mechanisms underlying cardiac fibrosis and inflammation. By targeting this pathway, novel therapies could be developed to improve cardiac diseases. Future research will be essential to validate these findings and translate them into clinical practice.

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Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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.

Author contributions: (I) Conception and design: Dongji Cui, Ying Xia, Tianhang Ding;(II) Administrative support: Dongji Cui, Baojun Zhang,Dongbo Li;(III) Provision of study materials or patients: Dongji Cui, Liyan Zeng, Tianhang Ding;(IV) Collection and assembly of data: Dongji Cui, Ying Xia, Tianhang Ding;(V) Data analysis and interpretation: Dongji Cui, Ying Xia, Liyan Zeng, Dongbo Li;(VI) Manuscript writing: All authors;(VII) Final approval of manuscript: All authors.

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