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MicroRNA-325 alleviates myocardial fibrosis after myocardial infarction via downregulating GLI1

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Abstract. – OBJECTIVE: To explore the role of microRNA-326 in myocardial fibrosis after myocardial infarction (MI) and its underlying mechanism.

MATERIALS AND METHODS: MI rat model was constructed via left anterior descending artery (LAD) ligation. Infarct tissues, infarct border zone tissues and remote zone tissues were harvested at postoperative 1st, 2nd, and 4th week, respectively. The mRNA levels of microRNA-325, collagen I, fibronectin, and transforming growth factor-β1 (TGF-β1) in the above tissues were detected by qRT-PCR (quantitative Real-time polymerase chain reaction). *In vivo* microRNA-325 upregulation was achieved by myocardial injection of microRNA-325 lentivirus. The effect of overexpressed microRNA-325 on overall survival (OS) and infarcted size was detected. Echocardiography was performed to evaluate rat cardiac function. Myocardial fibrosis affected by overexpressed microRNA-325 was evaluated via detecting α-SMA expression. Primary cardiac fibroblasts (CFs) were isolated and cultured *in vitro*. Cell counting kit-8 (CCK-8) and transwell assay were performed to evaluate the effect of microRNA-325 on regulating proliferative and migratory abilities of CFs. The regulatory role of microRNA-325 in GLI1 was verified by dual-luciferase reporter gene assay and Western blot.

RESULTS: MicroRNA-325 was downregulated in MI area, which was recovered to the normal level 4 weeks later. MicroRNA-325 overexpression could remarkably decrease the mortality and infarcted size of MI rats. Overexpressed microRNA-325 elevated LVEF and LVFS in MI rats. In vitro experiments demonstrated that microRNA-325 remarkably inhibited α-SMA expression, as well as proliferation and migration of CFs. Dual-luciferase reporter gene assay elucidated that microRNA-325 directly inhibited GLI1 expression.

CONCLUSIONS: Overexpressed microRNA-325 alleviates myocardial fibrosis after myocardial infarction via inhibiting GLI1 expression.

Key Words:
Myocardial infarction, Myocardial fibrosis, MicroRNA-325, GLI1.

Introduction

Acute myocardial infarction (MI) occurs after acute ischemic necrosis of myocardial tissue. MI is a clinical syndrome secondary to the rupture of coronary arteries and erosion, thrombosis and coronary artery occlusion, eventually resulting in ischemia, damage and necrosis of myocardial cells[1-3]. Although reperfusion therapy such as thrombolysis and percutaneous coronary intervention could markedly reduce myocardial cell death in MI patients, MI can further lead to myocardial remodeling[4]. Myocardial fibrosis after MI is one of the important steps in myocardial remodeling. The main pathological changes include cardiac fibroblast activation induced by ischemia and hyperplasia, and synthesis of large amounts of extracellular matrix (ECM). The above manifestations subsequently result in excessive deposition of ECM and changes in collagen composition. Based on the fibrosis pathogenesis, myocardial fibrosis is classified into alternative fibrosis and reactive fibrosis[5].

MicroRNAs are a class of endogenous, short-chain, non-coding RNAs with 18-22 nucleotides in length. Although microRNAs could not encode proteins due to the lack of open reading frame, they can degrade mRNA at transcriptional level by binding to the 3'UTR sequences of their target genes. Functionally, microRNAs regulate multiple biological processes, including cell proliferation, differentiation, apoptosis, and development[6]. In recent years, a large number of microRNAs have been shown to regulate various signaling

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pathways in myocardial fibrosis. For example, microRNA-208a has been shown to inhibit myocardial fibrosis and improve cardiac function *via* targeting endoglin[7]. MicroRNA-208ab regulates myocardial fibrosis after MI through the transcriptional factor GATA4[8]. MicroRNA-34a inhibits myocardial fibrosis in MI mice *via* regulating SMAD4[9]. MicroRNA-24 can improve myocardial fibrosis through transforming growth factor-β (TGF-β) pathway[10].

Li et al[11] have shown that differentially expressed microRNA-325 participates in the regulation of proliferation and migration of hematoma cells *via* targeting HMGB1. MicroRNA-325 is also differentially expressed in non-small cell lung cancer and cervical cancer[12,13]. Meanwhile, microRNA-325 enhances myocardial autophagy by binding to ARC, which exerts a crucial role in myocardial function homeostasis[14]. However, the role and mechanism of microRNA-325 in interstitial fibrosis after MI still needs further investigation.

Materials and Methods

MI Rat Model Construction

8-week-old male Sprague-Dawley (SD) rats weighing from 180-220 g were obtained from Department of Laboratory Animal Science, Peking University Health Science Center. MI rat model was constructed *via* left anterior descending artery (LAD) ligation[15]. Rats in sham group received the same procedures except for LAD ligation. Infarct tissues, infarct border zone tissues and remote zone tissues were harvested at postoperative 1st, 2nd, and 4th week, respectively. Myocardial tissues were immediately preserved in liquid nitrogen. The study was approved by The Second Hospital of Dalian Medical University Ethics Committee.

In Vivo Lentivirus Transfection

Rats were observed for 20 min after LAD ligation. When smooth breath and regular rhythm of rats were recovered, 1×10^7 TU LV-MicroRNA-325 and 1×10^7 TU LV-Vector per rat were injected into the myocardium 2 mm away from the infarcted area.

RNA Extraction and qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction)

Total RNA was extracted from the tissues and cells using the TRIzol kit (Invitrogen, Carlsbad, CA, USA), respectively, followed by measurement of RNA concentration using an ultraviolet spectrophotometer (Hitachi, Tokyo, Japan). The complementary Deoxyribose Nucleic Acid (cDNA) was synthesized according to the instructions of the miScriptRT II Kit. QRT-PCR was then performed based on the instructions of miScript SYBR Green PCR Kit. The relative expression level of the target gene was expressed by $2^{-\Delta\Delta Ct}$. Primers used in the study were as follows: Collagen I, F: TTCACCTACAGCACGCTTG, R: TTGGGATGGAGGGAGTTTAC; α-SMA, F: AGCCAGTCGCCATCAGGAAC, R: CCGGAGCCATTGTCACACAC; Fibronectin, F: ATGTGGACCCCTCCTGATAGT, R: GCCCAGTGATTTCAGCAAAGG; TGF-β1, F: TGACGTCACTGGAGTTGTACGG; R: GGTTCATGTCATGGATGGTGC; Collagen I α2, F: GCTTTGTGGATACGCGAACTC, R: CCAGCATTGGCATGTTGCT; Collagen III, F: GTTCGTGACCGTGACCTCG, R: TCTTGTCCTTGGGGTTCTTGC; GLI1, F: GGAGGACCTGCGGCTGACTGTGTAA, R: TGCTGTGCCTGTGGTCATCCTGATT; MicroRNA-325, F: GCCATTTCCTACAGCAACTC, R: CCATTTACATTCACCATCTTC.

Measurement of MI Areas

Briefly, mice were anaesthetized 24 h after LAD ligation. 1 mL of Evans blue dye (2.0% solution; Sigma-Aldrich, St. Louis, MO, USA) was injected into the vena cava to define the ischemic zone. Hearts were rapidly sectioned into five slices. The slices were incubated in 1.0% TTC (Sigma-Aldrich, St. Louis, MO, USA) for 15 min at 37°C. The infarcted area was defined as TTC-unstained area (white color). Infarct size was measured and calculated as the percentage of MI compared with the AAR[16].

Cell Culture and Transfection

Briefly, rats were anaesthetized with 1.0% isoflurane and sacrificed. The hearts were dissected, minced, and digested to prepare for cell suspension. Cells were then pre-plated for 1 h. The resultant cell suspension was changed[17]. Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (fetal bovine serum), 100 U/mL penicillin and 100 μg/mL streptomycin (HyClone, South Logan, UT, USA). Cells were maintained in a 5% CO_2 incubator at 37°C. Culture medium was replaced every two days.

Cell transfection was performed when the confluence was up to 70% according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). LV-MicroRNA-325 and LV-Vector were constructed by Gene Pharma (Shanghai, China).

Cell Counting Kit-8 (CCK-8) Assay

Transfected cells were seeded into 96-well plates at a density of 2×10^3/μL. 10 μL of CCK-8 solution (Cell Counting Kit-8, Dojindo, Kumamoto, Japan) were added in each well after cell adherence. The absorbance at 450 nm of each sample was measure by a microplate reader (Bio-Rad, Hercules, CA, USA). Each group had 6 replicates.

Transwell Assay

Transfected cells were prepared for cell suspension at a density of 1×10^6/mL. For transwell assay, 100 μL of cell suspension and 500 μL of DMEM containing 10% fetal bovine serum (FBS) were added into the upper and lower chamber, respectively. After cell culture for 10 h, cells were washed with PBS (phosphate-buffered saline), fixed with 80% ethanol and stained with crystal violet. Transwell chamber was observed and captured using an inverted microscope (Nikon, Tokyo, Japan).

Dual-Luciferase Reporter Gene Assay

Wild-type GLI (GLI1-WT) and mutant-type GLI (GLI1-MUT) were constructed based on psiCHECK2. Cells in logarithmic growth were seeded in the 24-well plates at a density of 4×10^5 per well. MicroRNA-325 mimic or negative control and GLI1-WT or GLI2-MUT were co-transfected in cells for 24 h. Luciferase activity was detected based on the instructions of Dual-Luciferase Reporter Assay System Kit (Promega, Madison, WI, USA).

Echocardiography

Rats were anesthetized and chest hairs were removed. Vevo2100 Image system was used for acquiring transthoracic echocardiographic images (VisualSonics Inc., Toronto, Canada). Two-dimensional parasternal short-axis images were measured at the level of the mid-papillary muscle, and M-mode tracings were recorded. LVESD (left ventricular end systolic dimension), LVEDD (left ventricular end diastolic dimension), LVEF (left ventricular ejection fraction) and LVFS (left ventricular fractional shortening) of each rat were recorded.

Western Blot

Total protein was extracted from treated cells by radioimmunoprecipitation assay (RIPA) solution (Beyotime, Shanghai, China). Protein sample was separated by electrophoresis on 10% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and then transferred to PVDF (polyvinylidene difluoride) membrane (Roche, Basel, Switzerland). After membranes were blocked with skimmed milk, the membranes were incubated with primary antibodies (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. The membranes were then washed with TBST (Tris-buffered Saline with Tween 20) and followed by the incubation of secondary antibody at room temperature for 1 h. The protein blot on the membrane was exposed by enhanced chemiluminescence (ECL).

Statistical Analysis

Prism 7.0 statistical software (La Jolla, CA, USA) was used for data analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared using the t-test. $p < 0.05$ considered the difference was statistically significant.

Results

MicroRNA-325 was Downregulated After MI

MI rat model was constructed *via* LAD ligation. MicroRNA-325 was downregulated in infarct tissues, infarct border zone tissues and remote zone tissues of MI rats compared with rats of sham group. The expression level of microRNA-325 was gradually elevated and restored to the normal level 4 weeks later (Figure 1A). Meanwhile, the expression levels of collagen I, fibronectin, and TGF-β1 were upregulated within the first week, gradually decreasing in the long period (Figure 1B-1D). The data demonstrated that there may be a close correlation between microRNA-325 and myocardial fibrosis after MI.

Overexpressed microRNA-325 Improved Cardiac Function After MI

MicroRNA-325 expression was upregulated *via* myocardial injection of lentivirus (Figure 2A). Overexpressed microRNA-325 remarkably decreased the mortality and infarct size of MI rats (Figure 2B and 2C). Echocardiography was performed after MI rat model

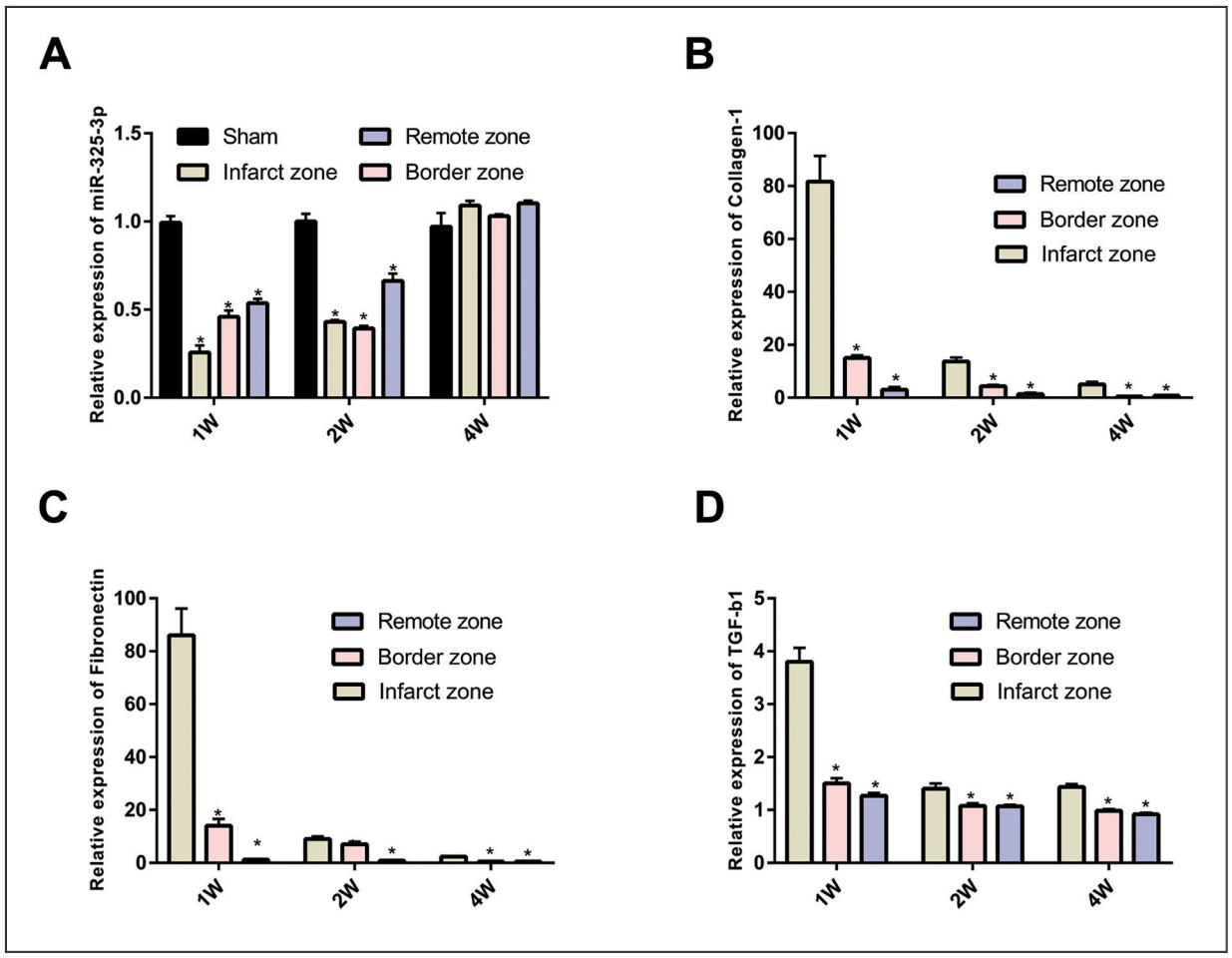

Figure 1. MicroRNA-325 was downregulated after MI. ***A,*** The expression level of microRNA-325 was gradually elevated and restored to the normal level 4 weeks later. ***B-D,*** Expression levels of collagen I (***B***), fibronectin (***C***) and TGF-β1 (***D***) were upregulated within the first week, whereas gradually decreased since after.

construction for 2 weeks. The results elucidated that LVEF and LVFS in MI rats were remarkably improved after microRNA-325 overexpression (Figure 2D and 2E). Furthermore, we speculated that infarct size decline is the result of decreased apoptosis and fibrosis of normal myocardium. The data showed that there was no significant difference in INF/AAR (infarct range/area at risk) between rats transfected with LV-MicroRNA-325 and LV-Vector (Figure 2F).

Overexpressed microRNA-325 Improved Myocardial Fibrosis After MI

To evaluate the effect of microRNA-325 on regulating myocardial fibrosis, cardiac tissues were harvested after MI rat model construction for 2 weeks. The data revealed that microRNA-325 overexpression remarkably downregulated collagen I and α-SMA (Figure 3A). The mRNA levels of collagen I α2, collagen III, fibronectin, and α-SMA were also decreased after microRNA-325 overexpression (Figure 3B). Subsequently, CFs were isolated and transfected with LV-MicroRNA-325. *In vitro* transfection efficacies of LV-MicroRNA-325 and LV-Vector were verified by qRT-PCR (Figure 3C). Similar to those of *in vivo* results, overexpressed microRNA-325 decreased the expressions of collagen I and α-SMA in CFs (Figure 3D). Proliferative and migratory abilities of CFs were also inhibited by LV-MicroRNA-325 transfection (Figure 3E and 3F), indicating that microRNA-325 participates in myocardial fibrosis *via* regulating cell proliferation and migration.

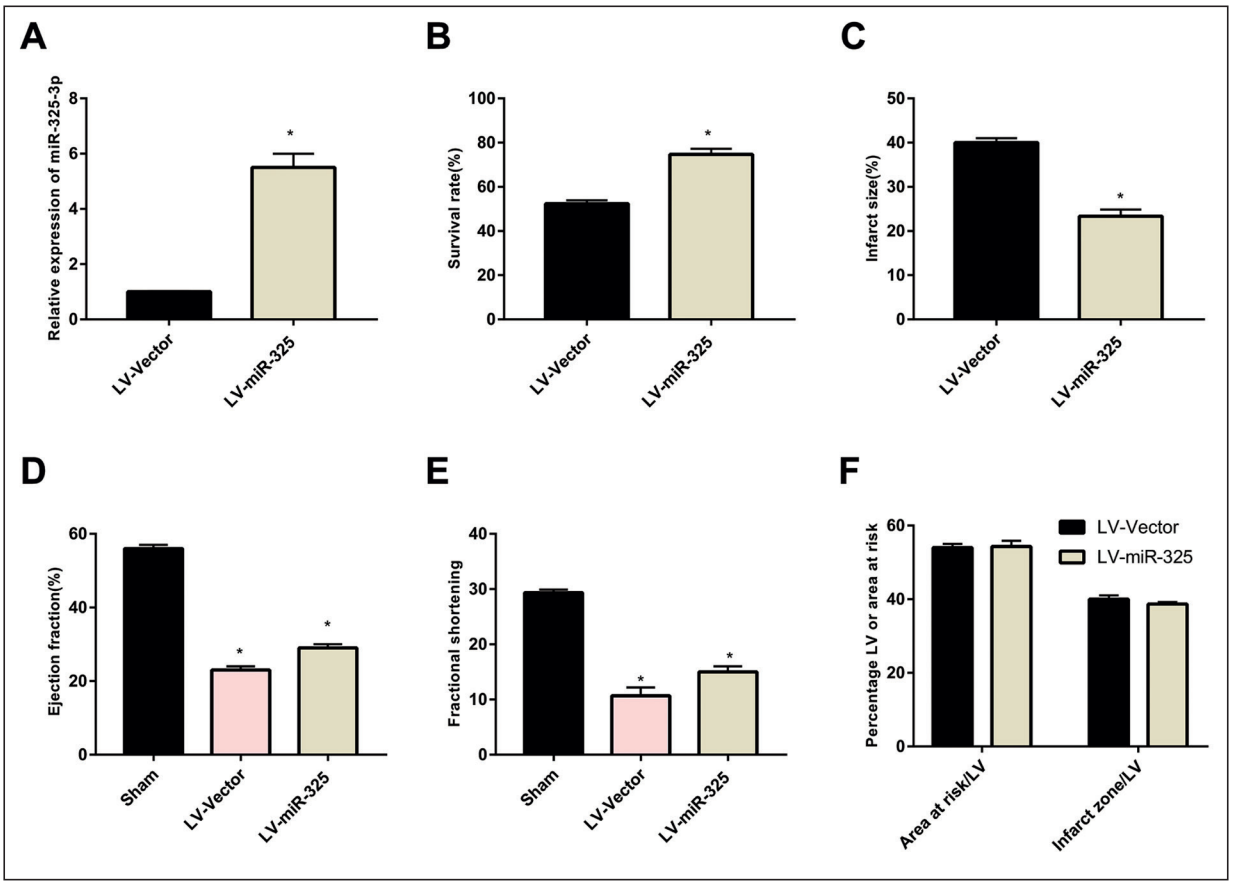

Figure 2. Overexpressed microRNA-325 improved cardiac function after MI. ***A,*** MicroRNA-325 expression was upregulated *via* myocardial injection of lentivirus. ***B, C,*** Overexpressed microRNA-325 remarkably decreased the mortality (***B***) and infarct size (***C***) of MI rats. ***D, E,*** LVEF (***D***) and LVFS (***E***) in MI rats were remarkably improved after microRNA-325 overexpression. ***F,*** There was no significant difference in INF/AAR between rats transfected with LV-MicroRNA-325 and LV-Vector.

GLI1 Was the Target Gene of microRNA-325

The target gene of microRNA-325 was predicted by bioinformatics and GLI1 was screened out. Differentially expressed GLI1 has been previously proved to be related to fibrosis[18]. Dual-luciferase reporter gene assay demonstrated that microRNA-325 remarkably decreased the luciferase activity of GLI1-WT than that of GLI1-MUT, indicating that microRNA-325 directly binds to GLI1 (Figure 4A and 4B). Both mRNA and protein levels of GLI1 were inhibited by microRNA-325 overexpression (Figure 4C and 4D), further indicating that microRNA-325 negatively regulates GLI1.

Discussion

Heart interstitial fibrosis is one of the crucial pathological processes of cardiac remodeling after MI, which is involved in the process of impaired myocardial contractility and failure[19]. Current studies have found that early myocardial fibrosis after MI is a favorable pathological process that is conducive to the healing of scar tissue and prevention of ventricular rupture. However, fibrosis in the infarct border zone after MI is an unfavorable pathological process that promotes cardiac remodeling, cardiac compliance decline and even heart failure[15]. Therefore, it is of great significance to prevent cardiac structure and preserve cardiac function after MI.

In the present work, microRNA-325 was downregulated in infarct size within the first week after MI. Downregulated microRNA-325 was beneficial to collagen fibers deposition in the infarct area, scar tissue formation and inhibition of infarct area expansion. Afterwards, microRNA-325 expression was gradually elevated and restored to the normal level at the 4th week, indi-

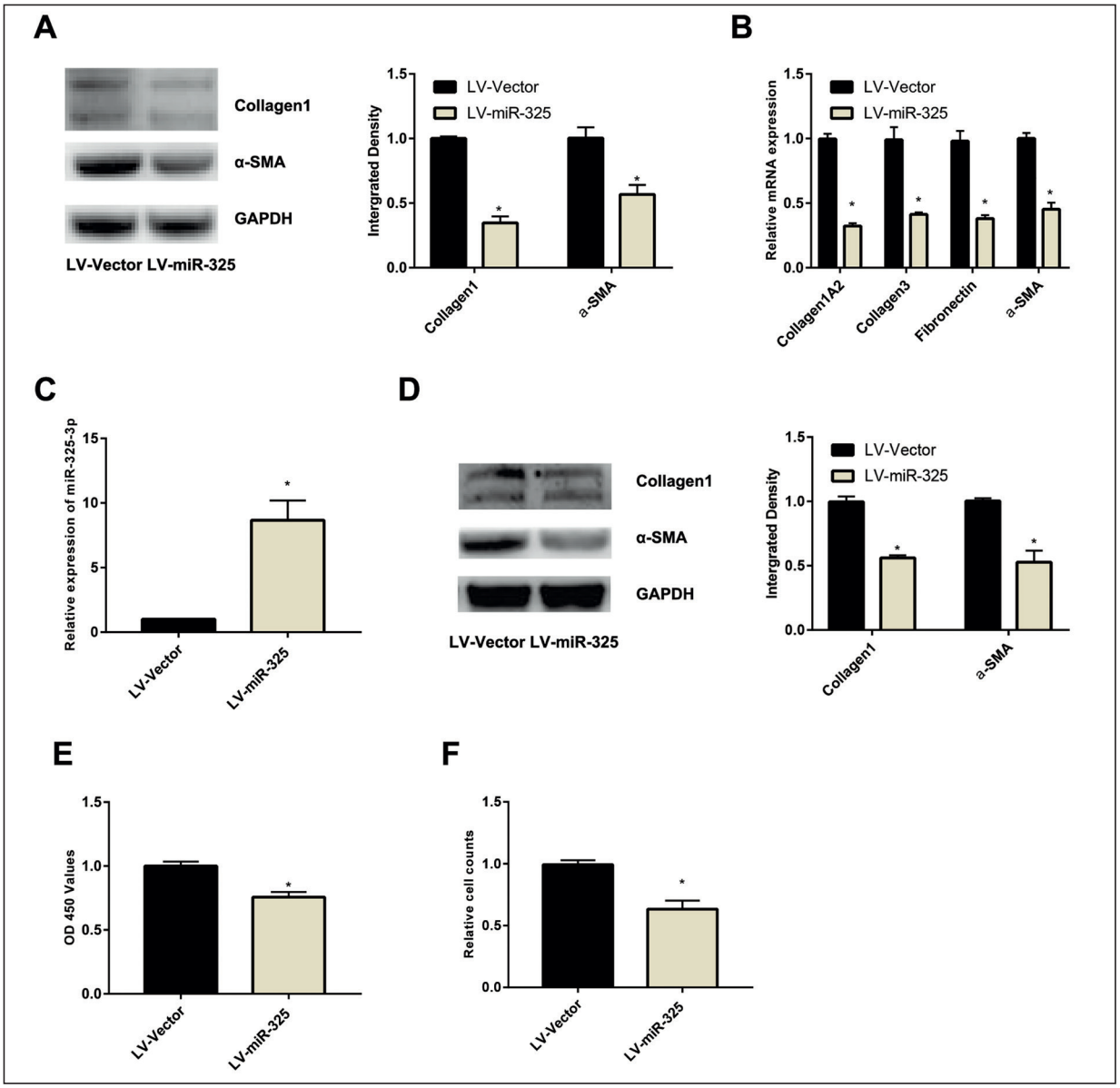

Figure 3. Overexpressed microRNA-325 improved myocardial fibrosis after MI. ***A,*** MicroRNA-325 overexpression remarkably downregulated collagen I and α-SMA. ***B,*** The mRNA levels of collagen I α2, collagen III, fibronectin and α-SMA were decreased after microRNA-325 overexpression. ***C,*** *In vitro* transfection efficacies of LV-MicroRNA-325 and LV-Vector were verified by qRT-PCR. ***D,*** Overexpressed microRNA-325 decreased expressions of collagen I and α-SMA in CFs. ***E, F,*** Proliferative (***E***) and migratory (***F***) abilities of CFs were inhibited by LV-MicroRNA-325 transfection.

cating that microRNA-325 may be the favorable factor for inhibiting myocardial fibrosis in infarct border zone.

Myocardial fibrosis is mainly characterized by the excessive accumulation of ECM, such as collagen fibers. During the process of myocardial fibrosis, myocardial fibroblasts are activated, proliferated and transformed into myofibroblasts expressing α-SMA[20,21]. Myofibroblasts further synthesize and secrete large amounts of ECM proteins, providing a structural framework for cardiomyocytes and inhibiting the spread of cardiac electrical activity. Collagen I and collagen III are the two main structural proteins of ECM. α-SMA is the typical marker for the conversion of cardiac fibroblasts into myofibroblasts. Therefore, collagen I α2, collagen III and α-SMA are often served as indicators of myocardial fibrosis[22,23].

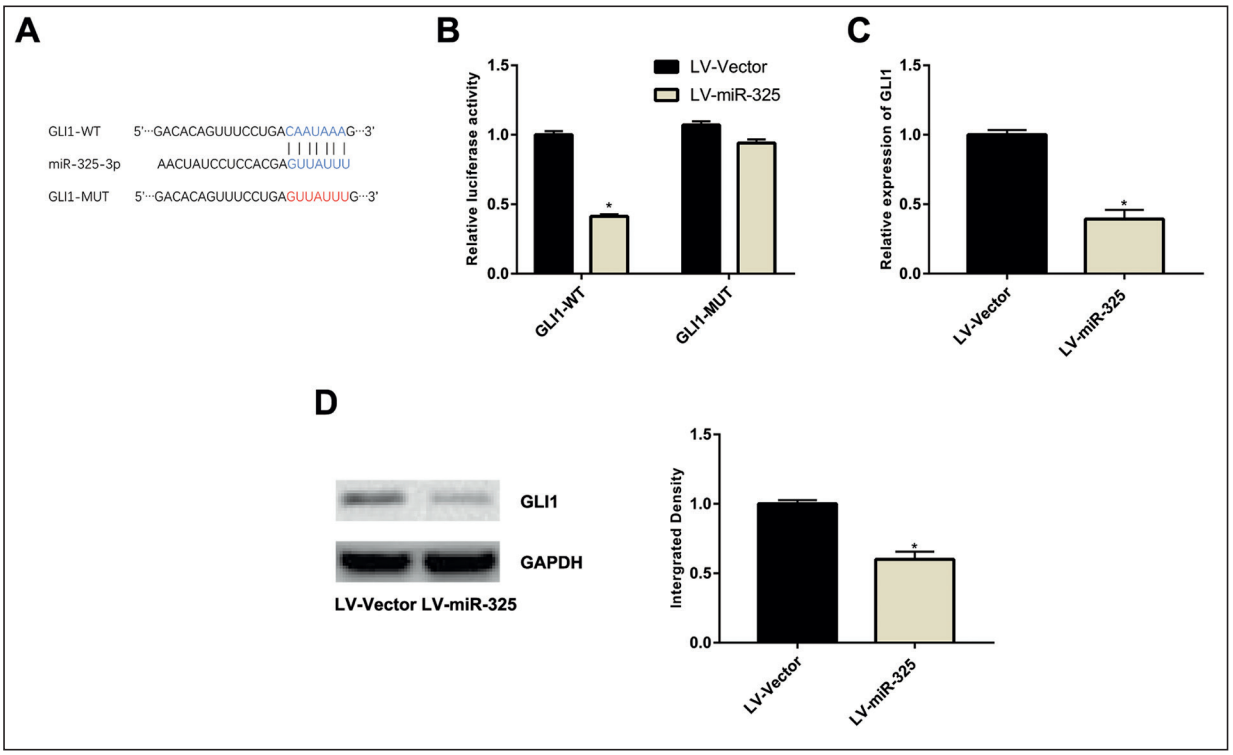

Figure 4. GLI1 was the target gene of microRNA-325. ***A, B,*** MicroRNA-325 remarkably decreased the luciferase activity of GLI1-WT than that of GLI1-MUT. ***C, D,*** Both mRNA (***C***) and protein (***D***) levels of GLI1 were inhibited by microRNA-325 overexpression.

Our results suggested that microRNA-325 overexpression remarkably decreased the elevated collagen I α2, collagen III and α-SMA in MI tissues, indicating the anti-fibrosis effect of microRNA-325.

GLI1 is inactive in mature normal tissues. Abnormally expressed GLI1 often leads to different types of diseases such as tumors and organ tissue fibrosis. Authors[24,25] have found that GLI1 is overexpressed in the formation and development of many organ fibrosis. For example, GLI1 is abnormally activated in an animal model of pancreatic cancer fibrosis. Overexpressed smoothened promotes fibrosis of pancreatic cancer through regulating GLI1 *via* Sonic Hedgehog pathway[26]. In this study, microRNA-325 could directly bind to GLI1 and degrade its expression, indicating the potential role of GLI1 in myocardial fibrosis.

Conclusions

We showed that overexpressed microRNA-325 alleviates myocardial fibrosis after myocardial infarction *via* inhibiting GLI1 expression.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Schmitt J, Duray G, Gersh BJ, Hohnloser SH. Atrial fibrillation in acute myocardial infarction: a systematic review of the incidence, clinical features and prognostic implications. Eur Heart J 2009; 30: 1038-1045.
2) Pedersen LR, Frestad D, Michelsen MM, Mygind ND, Rasmusen H, Suhrs HE, Prescott E. Risk factors for myocardial infarction in women and men: a review of the current literature. Curr Pharm Des 2016; 22: 3835-3852.
3) Zhang S, Cui R. The targeted regulation of miR-26a on PTEN-PI3K/AKT signaling pathway in myocardial fibrosis after myocardial infarction. Eur Rev Med Pharmacol Sci 2018; 22: 523-531.
4) Ayanian JZ, Guadagnoli E, McNeil BJ, Cleary PD. Treatment and outcomes of acute myocardial infarction among patients of cardiologists and generalist physicians. Arch Intern Med 1997; 157: 2570-2576.
5) Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction:

From inflammation to fibrosis. Circ Res 2016; 119: 91-112.

6) Pasquinelli AE, Ruvkun G. Control of developmental timing by microrna and their targets. Annu Rev Cell Dev Biol 2002; 18: 495-513.

7) Shyu KG, Wang BW, Cheng WP, Lo HM. MicroRNA-208a increases myocardial endoglin expression and myocardial fibrosis in acute myocardial infarction. Can J Cardiol 2015; 31: 679-690.

8) Zhou C, Cui Q, Su G, Guo X, Liu X, Zhang J. MicroRNA-208b alleviates post-infarction myocardial fibrosis in a rat model by inhibiting GATA4. Med Sci Monit 2016; 22: 1808-1816.

9) Huang Y, Qi Y, Du JQ, Zhang DF. MicroRNA-34a regulates cardiac fibrosis after myocardial infarction by targeting Smad4. Expert Opin Ther Targets 2014; 18: 1355-1365.

10) Wang J, Huang W, Xu R, Nie Y, Cao X, Meng J, Xu X, Hu S, Zheng Z. MicroRNA-24 regulates cardiac fibrosis after myocardial infarction. J Cell Mol Med 2012; 16: 2150-2160.

11) Li H, Huang W, Luo R. The microRNA-325 inhibits hepatocellular carcinoma progression by targeting high mobility group box 1. Diagn Pathol 2015; 10: 117.

12) Arora S, Ranade AR, Tran NL, Nasser S, Sridhar S, Korn RL, Ross JT, Dhruv H, Foss KM, Sibenaller Z, Ryken T, Gotway MB, Kim S, Weiss GJ. MicroRNA-328 is associated with (non-small) cell lung cancer (NSCLC) brain metastasis and mediates NSCLC migration. Int J Cancer 2011; 129: 2621-2631.

13) Liu X, Chen Z, Yu J, Xia J, Zhou X. MicroRNA profiling and head and neck cancer. Comp Funct Genomics 2009: 837514.

14) Bo L, Su-Ling D, Fang L, Lu-Yu Z, Tao A, Stefan D, Kun W, Pei-Feng L. Autophagic program is regulated by miR-325. Cell Death Differ 2014; 21: 967-977.

15) van den Borne SW, Diez J, Blankesteijn WM, Verjans J, Hofstra L, Narula J. Myocardial remodeling after infarction: the role of myofibroblasts. Nat Rev Cardiol 2010; 7: 30-37.

16) Kurrelmeyer KM, Michael LH, Baumgarten G, Taffet GE, Peschon JJ, Sivasubramanian N, Entman ML, Mann DL. Endogenous tumor necrosis factor protects the adult cardiac myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. Proc Natl Acad Sci U S A 2000; 97: 5456-5461.

17) Chen J, Shearer GC, Chen Q, Healy CL, Beyer AJ, Nareddy VB, Gerdes AM, Harris WS, O'Connell TD, Wang D. Omega-3 fatty acids prevent pressure overload-induced cardiac fibrosis through activation of cyclic GMP/protein kinase G signaling in cardiac fibroblasts. Circulation 2011; 123: 584-593.

18) Moshai EF, Wemeau-Stervinou L, Cigna N, Brayer S, Somme JM, Crestani B, Mailleux AA. Targeting the hedgehog-glioma-associated oncogene homolog pathway inhibits bleomycin-induced lung fibrosis in mice. Am J Respir Cell Mol Biol 2014; 51: 11-25.

19) Parikh A, Patel D, McTiernan CF, Xiang W, Haney J, Yang L, Lin B, Kaplan AD, Bett GC, Rasmusson RL, Shroff SG, Schwartzman D, Salama G. Relaxin suppresses atrial fibrillation by reversing fibrosis and myocyte hypertrophy and increasing conduction velocity and sodium current in spontaneously hypertensive rat hearts. Circ Res 2013; 113: 313-321.

20) Shinde AV, Frangogiannis NG. Fibroblasts in myocardial infarction: a role in inflammation and repair. J Mol Cell Cardiol 2014; 70: 74-82.

21) Talman V, Ruskoaho H. Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration. Cell Tissue Res 2016; 365: 563-581.

22) Fan GP, Wang W, Zhao H, Cai L, Zhang PD, Yang ZH, Zhang J, Wang X. Pharmacological inhibition of focal adhesion kinase attenuates cardiac fibrosis in mice cardiac fibroblast and post-myocardial-Infarction models. Cell Physiol Biochem 2015; 37: 515-526.

23) Zhong J, Basu R, Guo D, Chow FL, Byrns S, Schuster M, Loibner H, Wang XH, Penninger JM, Kassiri Z, Oudit GY. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. Circulation 2010; 122: 717-728, 18-728.

24) Ding H, Zhou D, Hao S, Zhou L, He W, Nie J, Hou FF, Liu Y. Sonic hedgehog signaling mediates epithelial-mesenchymal communication and promotes renal fibrosis. J Am Soc Nephrol 2012; 23: 801-813.

25) Kimura H, Stephen D, Joyner A, Curran T. Gli1 is important for medulloblastoma formation in Ptc1+/- mice. Oncogene 2005; 24: 4026-4036.

26) Walter K, Omura N, Hong SM, Griffith M, Vincent A, Borges M, Goggins M. Overexpression of smoothened activates the sonic hedgehog signaling pathway in pancreatic cancer-associated fibroblasts. Clin Cancer Res 2010; 16: 1781-1789.