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CircMACF1 alleviates myocardial fibrosis after acute myocardial infarction by suppressing cardiac fibroblast activation via the miR-16-5p/SMAD7 axis

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Abstract

Circular RNAs (circRNAs) played a pivotal role in myocardial fibrosis after acute myocardial infarction (AMI). The activation of cardiac fibroblasts (CFs) and accumulation of extracellular matrix are the main characteristics of myocardial fibrosis. In our research, we aimed to elucidate the functional roles of circMACF1 in CF activation after AMI as well as the underlying mechanism. Human CFs were activated by TGF-β1 treatment. qPCR and western blotting were performed to investigate gene and protein expression. CCK-8 and transwell assays were carried out to measure cell proliferation, and migration. Immunofluorescence was used to investigate α-SMA level. The interaction between miR-16-5p and circMACF1 or SMAD7 was revealed by RIP or dual luciferase reporter gene assays. CircMACF1 and SMAD7 were repressed in AMI patients and CFs treated with TGF-β1, and miR-16-5p was increased. In addition, circMACF1 was resistant to RNase R and abundantly expressed in the cytoplasm. Overexpression of circMACF1 inhibited cell proliferation and migration and reduced the expression levels of fibrosis-related proteins, including Collagen I, Collagen III, and α-SMA. Furthermore, circMCAF1 could directly bind to miR-16-5p, and SMAD7 was a target gene of miR-16-5p. Knockdown of miR-16-5p suppressed the activation, proliferation, and migration of TGF-β1-treated CFs, but silencing circMACF1 or SMAD7 partially reversed this phenomenon. CircMACF1 attenuated the TGF-β1-induced activation, proliferation and migration of CFs via the miR-16-5p/SMAD7 signaling pathway, indicating that circMACF1 might be a new therapeutic target for AMI.

Abbreviations: AMI = acute myocardial infarction, Ang II = angiotensin II, CCK-8 = cell counting kit-8, CFs = cardiac fibroblasts, circMACF1 = circular microtubule actin crosslinking factor 1, circRNAs = circular RNAs, ECM = extracellular matrix, qPCR = real time fluorescent quantitative PCR, RIP = RNA immunoprecipitation, SMAD7 = mothers against decapentaplegic homolog 7, TGF = transforming growth factor.

Keywords: acute myocardial infarction, cardiac fibroblast activation, circMACF1, miR-16-5p, SMAD7

1. Introduction

Acute myocardial infarction (AMI) the leading cause of heart failure.[1,2] Fibrosis is one of the major factors involved in the development of most cardiovascular diseases, which can lead to structural changes of myocardium and vascular wall.[3] Myocardial fibrosis refers to the formation of cardiac structure formed via the accumulation of extracellular matrix (ECM) and increased activity of cardiac fibroblasts (CFs), which contributes to a variety of heart diseases including MI.[4] Therefore, it is necessary to explore the molecular mechanism underlying myocardial fibrosis and to develop new interventions to inhibit ECM deposition and the activation of CFs for the treatment of AMI and other heart diseases.

Circular RNAs (circRNAs), which are non-coding RNAs, perform key regulatory functions in a variety of diseases and play pivotal roles in regulating cell metabolism by binding to their corresponding miRNAs or directly interacting with proteins.[5] Increasing evidence has demonstrated that circRNAs participate in the development of a variety of sickness, including cardiovascular disease,[6] cancer,[7] osteoarthritis.[8] A study showed that overexpression of circHIPK3 could reduce heart failure and myocardial fibrosis after MI.[9] Circular microtubule actin crosslinking factor 1 (circMACF1) is considered a potential biomarker of cardiovascular disease, moreover, circMACF1 expression is significantly decreased in mice with AMI.[10,11] However, the functional roles of circMACF1 in CF activation after AMI remain unclear.

Informed consent was obtained from the study participants.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

All the patients and their families provided consent, and all the experiments were approved by the ethics committee of The Fourth Hospital of Changsha.

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MiRNAs are non-coding regulatory RNAs that are17-25 nt in length. After transcription, miRNAs can regulate mRNA levels by binding to the 3'untranslatable region of target mRNAs.[12] The abnormally expressed miRNAs perform a key function in cell differentiation or cell cycle regulation.[13] Previous reports have indicated that multiple miRNAs are important diagnostic or prognostic markers for different phases of cardiovascular disease progression.[14] Tanshinone IIA plays an antitumor role by inhibiting cell migration, invasion, and proliferation by regulating miR-16-5p.[15] In ischemia/reperfusion exposed human adult ventricular cardiomyocytes (AC16), miR-16-5p expression was markedly enhanced.[16] Nevertheless, the mechanism of miR-16-5p in myocardial fibrosis after AMI remains uninvestigated.

Mothers against decapentaplegic homolog 7 (SMAD7), a critical regulatory protein in transforming growth factor (TGF) pathway, plays pivotal roles in cell proliferation, differentiation and apoptosis.[17] The abnormal regulation of SMAD signaling pathway can lead to severe myocardial fibrosis in childhood and its fibrogenic effects persist into adulthood, which is a crucial factor in causing sudden cardiac death and heart failure in hypertrophic obstructive cardiomyopathy patients.[18]Previous study has clearly indicated that cell-specific approaches targeting the SMAD pathway may hold therapeutic promise in the context of myocardial infarction and heart failure.[19]In relevant reports, researchers have found that SMAD7 can inhibit myocardial fibroblast activation and prevent post-infarction heart failure by modulating the TGF-β/human epidermal growth factor receptor 2 pathway.[20]It was indicated that miR-21 accelerated myocardial fibrosis following MI via directly regulating SMAD7.[21] Moreover, SMAD7 expression has been found to be decreased in rats following MI.[22] However, the regulatory effect of circMACF1/miR-16-5p/SMAD7 signaling axis in myocardial fibrosis after AMI remains to be explored.

We elucidated the functions of circMACF1 in the activation of CFs after AMI. We showed that circMACF1 silencing suppressed the proliferation, and migration of CFs by down-regulating SMAD7 expression by directly targeting miR-16-5p and suppressing the levels of fibrosis-related proteins. These results suggested that circMACF1 could be a potential marker for AMI.

2. Materials and methods

2.1. Collection of clinical samples

Whole blood samples were collected from 35 AMI patients and 35 healthy people in the present study. All patients and their families provided consent, and all the experiments were approved by the ethics committee of The Fourth Hospital of Changsha.

2.2. Cell and culture treatment

Human CFs were obtained from ScienCell Research Laboratories (Carlsbad, CA) for further in vitro studies. The cells were incubated on Fibroblast Medium-2 (ScienCell) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and antibiotics. Subsequently, these cells were cultured in a humid incubator containing 5% CO_2 at 37 °C. To induce cell activation into myofibroblasts, TGF-β1 (10 ng/mL; Sigma, St. Louis, MO, USA) or angiotensin II (Ang II, 100 nM; Sigma) was incubated with cells for 48 hours.

The circMACF1 overexpression construction (circMACF1), small interfering RNA (si)-circMACF1, si-SMAD7, miR-16-5p mimics, miR-16-5p inhibitor and corresponding negative controls were synthetized by Genepharm (Shanghai, China). Then, the CFs were transfected utilizing Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA) for 48 hours according to the corresponding experimental conditions.

2.3. RNA preparation and qPCR

TRIzol (Invitrogen, Carlsbad, CA, USA) was used to collect total RNA from cells. Nuclear and cytoplasmic fractions were separated by utilizing a PARIS™ kit (Thermo Fisher Scientific). Then, total RNA was incubated at 40°C for 60 minutes with or without 3 U/μg RNase R (Epicentre Technologies, Madison, WI). Next, an RNeasy MinElute cleaning kit was used to purify the resulting RNA. A reverse transcription kit (Takara, Japan) was used to reverse transcribe cDNA. Using cDNA as a template, real time fluorescent quantitative PCR (qPCR) was performed for further analysis. The relative gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.4. Western blotting

The proteins were lysed with radioimmunoprecipitation assay buffer. Next, a bicinchoninic acid kit (Beyotime, Shanghai, China) was utilized to measure the protein concentration. Eight percent sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed to separate the same amount of protein from each sample, and the protein was then transferred onto polyvinylidene fluoride membranes. 5% bovine serum albumins in tris buffered saline with Tween 20 was used to block the membrances for 1 hour. The primary antibodies were added and incubated at 4°C overnight. Next, the membranes were incubated with the secondary antibody for 2 hours at room temperature. The bands were visualized with an enhanced chemiluminescence detection system and relative densities were quantified by image J software.

2.5. Cell proliferation

Cell counting kit-8 (CCK-8; Dojindo, Kumamoto, Japan) measure cell proliferation. Then, cells were plated in a 96-well microplate at a density of 3000 cells/well. According to the instructions, 10 μL CCK-8 solution was added to each well. Then, the plates were cultured in an incubator for 4 hours, and at 450 nm, the absorbance was measured.

2.6. Cell migration

Transwell assays were performed for the cell migration assessment. Cells were seeded in a 24-well microplate (Corning, NY) at a density of 2×10^5. Then, 600 μL of medium supplemented with 10% serum was added to the lower chamber, and, 200 μL of cell suspension was supplied to the upper chamber. Then, the cells were incubated in the incubator for 24 hours and stained with 0.1% crystal violet, and then counted under a microscope (Olympus Corporation, Tokyo, Japan).

2.7. Dual luciferase reporter gene assay

The binding sites between miR-16-5p and circMACF1 or SMAD7 were predicted by bioinformatics software. The target fragment was amplified and inserted into the PmirGLO vector (Promega, Madison, WI). CFs were cultured and seeded in a 6-well culture plate for 24 hours. Then, the cells were cotransfected with reporter gene plasmids (circMACF-WT/MUT and SMAD7-WT/MUT; WT: wild type, MUT: mutant) and miR-16-5p mimics or miR-NC, and then, the luciferase activity was investigated by dual-luciferase reporter assay system (Promega).

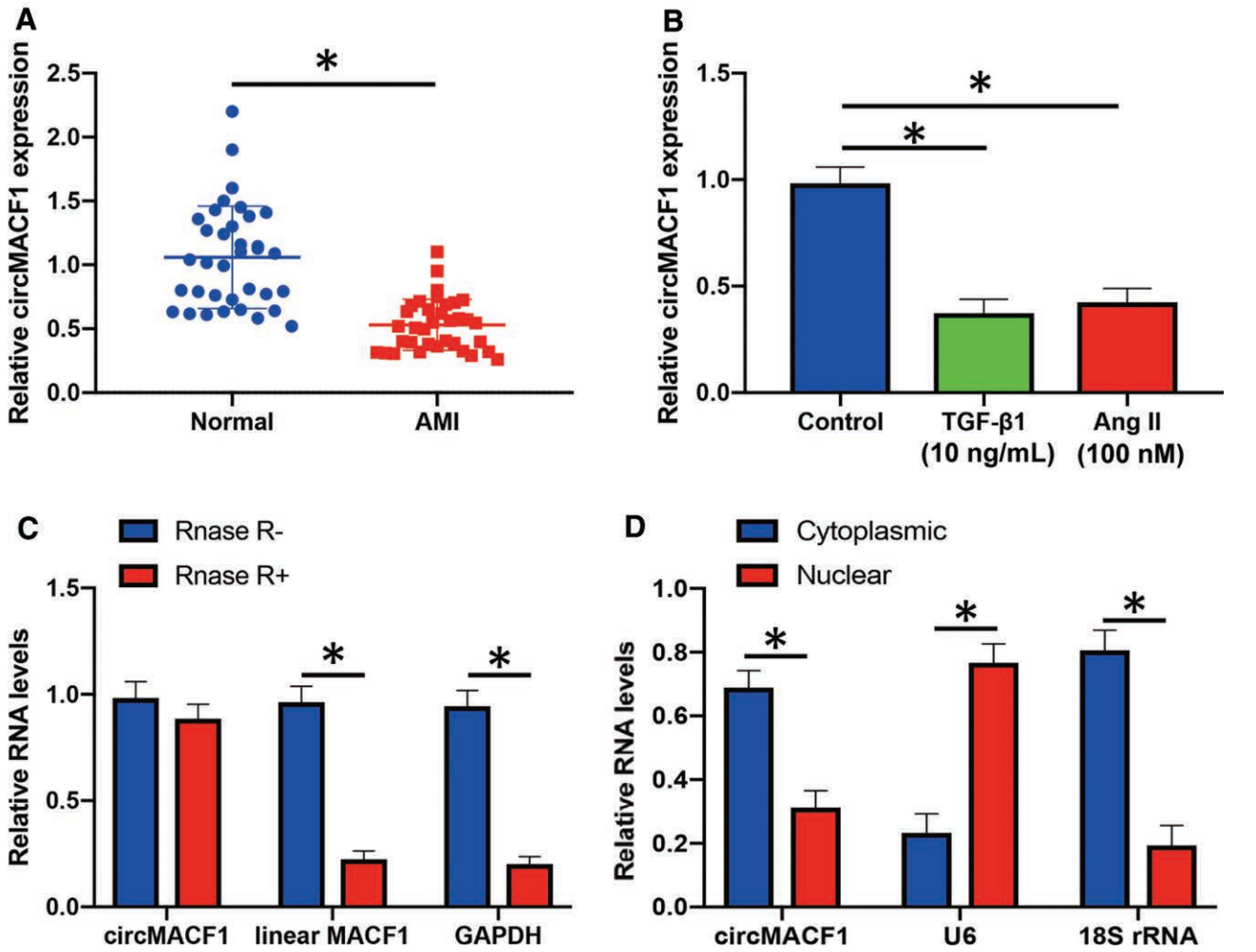

Figure 1. CircMACF1 was repressed in AMI tissues and activated CFs. The blood samples were collected from 35 AMI patients and 35 healthy people. (A) The expression of circMACF1 in AMI patients was assessed by using qPCR. CFs were treated with TGF-β1 (10 ng/mL) or Ang II (100 nM) for 48 hours. (B) CircMACF1 expression in activated CFs was examined by qPCR. The circMACF1 from CFs was treated with RNase R (3 U/μg) and incubated for 60 minutes at 40°C. (C) Resistance of circMACF1 to RNase R digestion was assessed via real-time PCR. (D) qPCR was used to measure the nuclear and cytoplasmic distribution of circMACF1. All the data are presented as the mean ± standard deviation. *$P < .05$. AMI = acute myocardial infarction, CFs = cardiac fibroblasts, circMACF1 = circular microtubule actin crosslinking factor 1, qPCR = real time fluorescent quantitative PCR, TGF = transforming growth factor.

2.8. Immunofluorescence

Cells were seeded on collagen-coated glass and cultured in medium at 37°C, in an incubator with 5% CO_2. The cells were washed with PBS, and then, the cells were fixed with 4% formaldehyde and permeabilized with 0.2% Triton X-100. Then, the primary antibody was added and incubated at 4°C overnight, followed by treatment with the secondary antibody. The cells were then modified by utilizing a confocal microscope (Nikon, Japan).

2.9. RNA immunoprecipitation (RIP)

The RIP experiment was conducted with the Magna RIP RNA Binding Protein Immunoprecipitation Kit (Millipore, MA). The cells (1×10^7) were digested using RIP lysis buffer, followed by incubation with magnetic beads conjugated with anti-Argonaute2 or IgG (negative control). Then, immunoprecipitated RNAs were extracted and qPCR assay was used to measure circMACF1 enrichment.

2.10. Statistical analysis

The data repeated 3 times independently, all data were represented as mean ± standard deviation. The data was analyzed using GraphPad Prism 8.0. The significant difference of 2 groups or multiple groups using Student *t* test or one way ANOVA. $P < .05$ was considered to have significant difference.

3. Results

3.1. CircMACF1 was downregulated in AMI patients and activated CFs

To explore the function of circMACF1 in AMI, qPCR assay was performed. The results indicated that circMACF1 was decreased in the AMI group compare with the normal group (Fig. 1A). Consistently, circMACF1 was downregulated in CFs treated with TGF-β1 or Ang II (Fig. 1B). Furthermore, the resistance of circMACF1 to RNase R treatment was detected (Fig. 1C). Subsequently, we investigated the expression of circMACF1 in the nucleus and cytoplasm and found that circMACF1 was abundantly expressed in the cytoplasm (Fig. 1D). In short, circMACF1 was lowly expressed in AMI and may have a pivotal function in the activation of CFs after AMI.

3.2. CircMACF1 overexpression suppressed the activation, proliferation and migration of CFs treated with TGF-β1

To identify the effect of circMACF1 in CF activation after AMI, we constructed the overexpression plasmid of circMACF1 and

transfected into TGF-β1 treated CFs. As indicated in Figure 2A, circMACF1 expression was markedly upregulated after transfection. To explore the regulatory effect of circMACF1 on the expression of myocardial fibrosis-associated proteins in CFs, collagen I, collagen III and α-SMA expression were measured via qPCR and western blot assays. The results displayed fibrosis-related protein levels were evidently enhanced by TGF-β1 treatment, and this effect was suppressed after circMACF1 upregulation (Fig. 2B and C). In addition, the activation of CFs was confirmed by immunofluorescence analysis, which revealed increased α-SMA expression in CFs treated with TGF-β1 (Fig. 2D). Furthermore, up-regulation of circMACF1 inhibited the cell proliferation (Fig. 2E) and migration (Fig. 2F) of activated CFs. Taken together, overexpression of circMACF1 could suppress cell migration, and proliferation of TGF-β1 induced CFs.

3.3. CircMACF1 acted as a sponge of miR-16-5p

Then, we analyzed the relationship of circMACF1 and miR-16-5p. In comparison with normal group, the level of miR-16-5p was enhanced in AMI patient (Fig. 3A). Moreover, we found that there was a negative regulatory relationship between circMACF1 and miR-16-5p in AMI (Fig. 3B). After transfection, miR-16-5p was dramatically elevated in the miR-16-5p mimics group but downregulated after treated with knockdown of miR-16-5p (Fig. 3C). According to bioinformatics software prediction, there were binding domains between circMACF1 and miR-16-5p (Fig. 3D). To identify whether circMACF1 acts as a sponge of miR-16-5p, the luciferase activity was detected. Results expounded that the expression of circMACF1 was preferentially enriched in the Argonaute2 immunoprecipitates, compared to the negative control IgG (Fig. 3E). After cotransfection with miR-16-5p mimics and circMACF1-WT, luciferase activity was markedly inhibited, while knockdown of miR-16-5p elevated the activity of luciferase when co-treated with circMACF1-WT (Fig. 3F). To reveal the regulation between miR-16-5p and circMACF1, we transfected circMACF1 silencing and circMACF1 overexpression plasmids into CFs. The silencing of circMACF1 could enhance miR-16-5p expression, while the overexpression of circMACF1 had the opposite trend (Fig. 3G and H). Collectively, circMACF1 could act as a sponge of miR-16-5p.

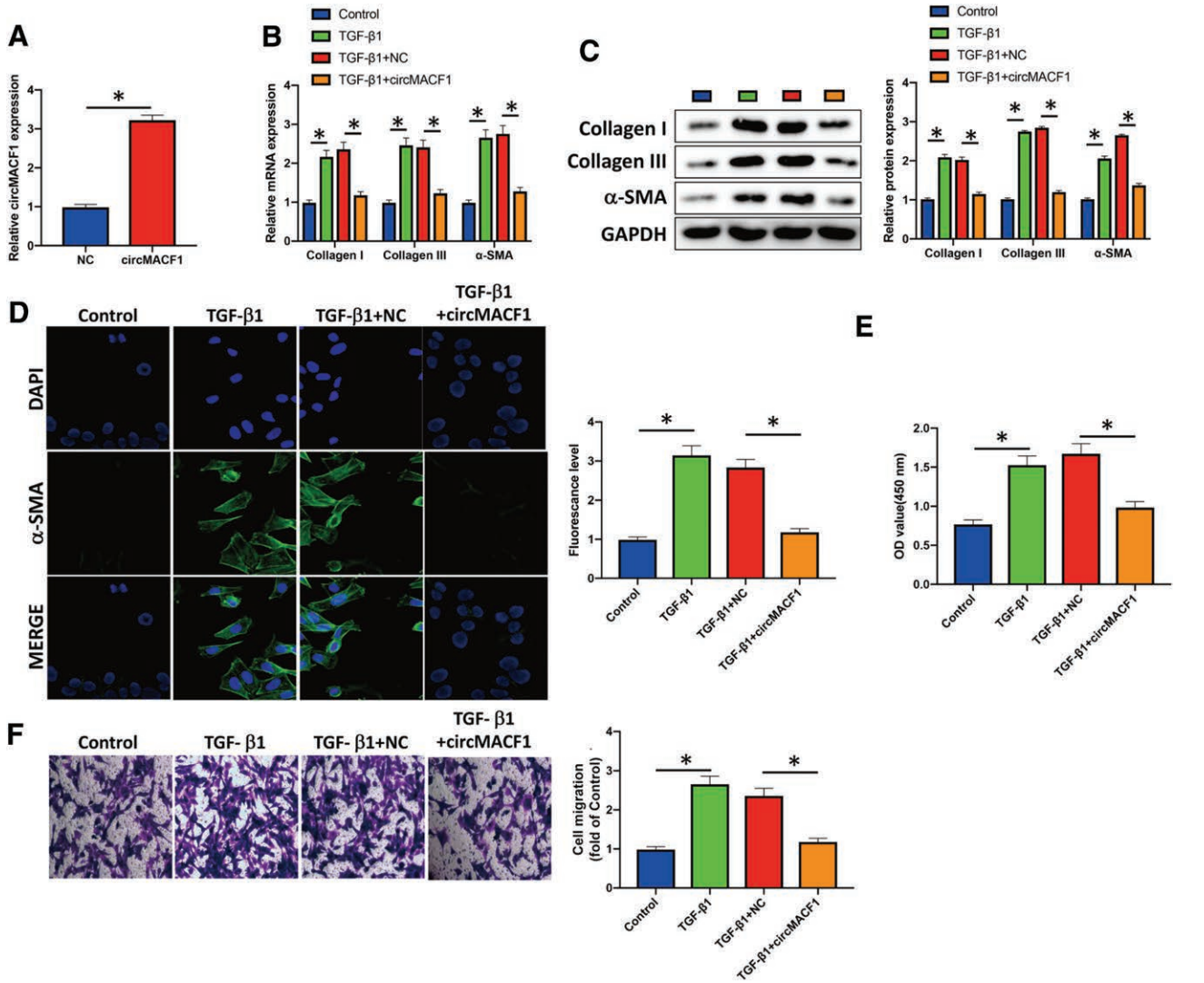

Figure 2. CircMACF1 overexpression suppressed the activation, proliferation and migration of TGF-β1-treated CFs. CFs were transfected with circMACF1 overexpression plasmid (100 nM) and its negative control (NC; 100 nM) for 48 hours. (A) qPCR was used to measure the level of circMACF1. CFs were transfected with NC and circMACF1 overexpression plasmids (100 nM) before the treatment with TGF-β1 (10 ng/mL). (B) Fibrosis-related protein expression levels were measured via qPCR. (C) The protein levels of fibrosis-related proteins were examined by western blotting assay. (D) Immunofluorescence was performed to investigate α-SMA expression. (E) CCK-8 was carried out to investigate cell viability. (F) Cell migration was investigated by Transwell assay. All the data are presented as the mean ± standard deviation. *$P < .05$. CFs = cardiac fibroblasts, circMACF1 = circular microtubule actin crosslinking factor 1, qPCR = real time fluorescent quantitative PCR, TGF = transforming growth factor.

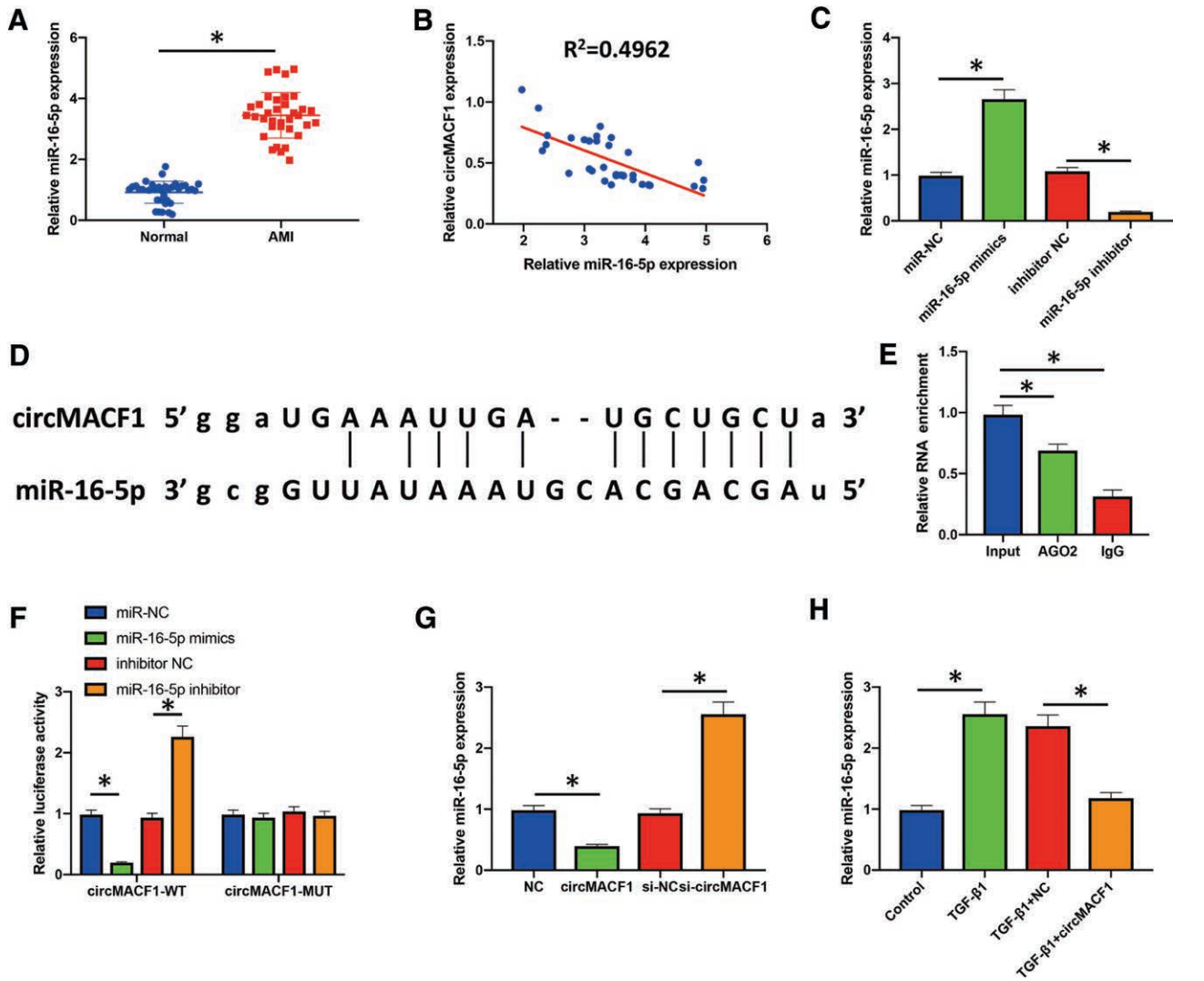

Figure 3. CircMACF1 acted as a sponge of miR-16-5p. The blood samples were collected from 35 AMI patients and 35 healthy people. (A) MiR-16-5p expression in AMI patients was measured by qPCR. (B) The correlation analysis of miR-16-5p and circMACF1 in AMI patients. CFs were transfected with miR-NC, miR-16-5p mimics (50 nM), inhibitor NC and miR-16-5p inhibitor (100 nM) for 48 hours. (C) MiR-16-5p expression in transfected CFs was detected by qPCR. (D) Bioinformatics software was used to predict the binding sites between miR-16-5p and circMACF1. (E) RIP confirmed the interaction of circMACF1 and miR-16-5p. (F) A dual luciferase reporter gene assay was used to measure the luciferase activity. CFs were transfected with circMACF1 ovexexpression plasmid (100 nM) or silencing plasmid (50 nM) for 48 hours. (G, H) qPCR was used to measure miR-16-5p expression without or with TGF-β1 treatment (10 ng/mL) in circMACF1 transfected cells. All the data are presented as the mean ± standard deviation. * $P < .05$. AMI = acute myocardial infarction, CFs = cardiac fibroblasts, circMACF1 = circular microtubule actin crosslinking factor 1, qPCR = real time fluorescent quantitative PCR, RIP = RNA immunoprecipitation, TGF = transforming growth factor.

3.4. SMAD7 could be a target gene of miR-16-5p

Subsequently, we explored the expression of SMAD7 in AMI patients, the results showed that SMAD7 was significantly enhanced in blood samples (Fig. 4A). Furthermore, there was a negative regulatory relationship between miR-16-5p and SMAD7 in patients with AMI (Fig. 4B and C). In addition, miR-16-5p bound to the SMAD7 3'untranslatable region (Fig. 4D). We cotransfected with miR-16-5p mimics/inhibitor and SMAD7-WT, which significantly inhibited/increased the luciferase activity, but no changes were observed in cells treated with SMAD7-MUT (Fig. 4E). Furthermore, miR-16-5p mimics suppressed the expression level of SMAD7, but SMAD7 expression increased after transfection with the miR-16-5p inhibitor (Fig. 4F and G). Furthermore, the circMACF1/miR-16-5p axis modulated SMAD7 expression in TGF-β1 stimulated CFs (Fig. 4H and I). Therefore, SMAD7 may be a target gene of miR-16-5p.

3.5. CircMACF1 inhibited CF activation, proliferation and migration by regulating the miR-16-5p/SMAD7 axis

To explicit the biofunction of circMACF1/miR-16-5p/SMAD7 pathway in CF activation, we would transfect miR-16-5p inhibition or SMAD7 knockdown into CFs treated by silencing circMACF1. qPCR and western blotting results showed SMAD7 expression was down-regulated after si-SMAD7 transfection (Fig. 5A and B). The fibrosis related proteins were downregulated by the miR-16-5p inhibitor, while downregulation of circMACF1 or SMAD7 partly abolished this effect (Fig. 5C and D). In the immunofluorescence assay, miR-16-5p downregulation inhibited the increase in α-SMA expression in TGF-β1 treated CFs, and silencing circMACF1 or SMAD7 enhanced the expression of α-SMA (Fig. 5E). Additionally, miR-16-5p repression significantly suppressed the cell proliferation, and migration in TGF-β1-treated CFs, while knockdown

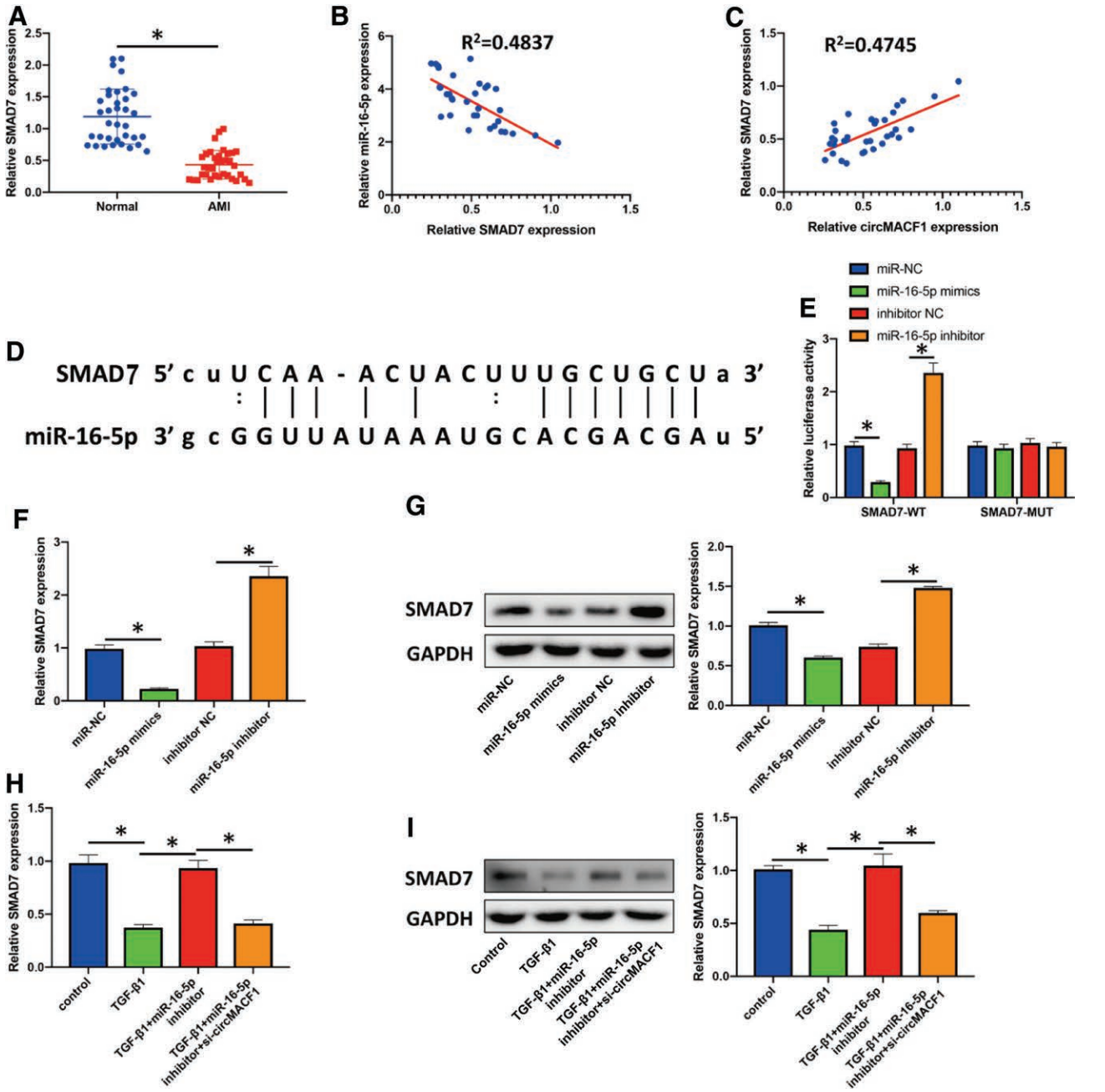

Figure 4. SMAD7 could be a target gene of miR-16-5p. The blood samples were collected from 35 AMI patients and 35 healthy people. (A) The expression of SMAD7 in AMI patients was investigated by qPCR. (B) MiR-16-5p was negatively correlated with SMAD7 in AIM. (C) CircMACF1 was positively correlated with SMAD7 in AMI. (D) Bioinformatics software predicted the binding sites between miR-16-5p and SMAD7. CFs were transfected with miR-16-5p mimics (50 nM) or miR-16-5p inhibitor (100 nM) for 48 hours. (E) Luciferase activity was examined via dual luciferase reporter gene assay. (F, G) The regulation of SMAD7 mRNA or protein levels by miR-16-5p were examined through qPCR or western blotting. CFs were transfected with miR-16-5p inhibitor (100 nM) and/or si-circMACF1 (50 nM) before the treatment with TGF-β1 (10 ng/mL) for 48 hours. (H, I) The mRNA level or protein expression of SMAD7 was investigated via qPCR or western blotting. All the data are presented as the mean ± standard deviation. *$P < .05$. AMI = acute myocardial infarction, CFs = cardiac fibroblasts, circMACF1 = circular microtubule actin crosslinking factor 1, qPCR = real time fluorescent quantitative PCR, SMAD7 = mothers against decapentaplegic homolog 7, TGF = transforming growth factor.

of circMACF1 or SMAD7 partially reversed this phenomenon (Fig. 5F and G). In summary, circMACF1 could inhibited CF activation, proliferation and migration induced by TGF-β1 through mediation miR-16-5p/SMAD7 signaling axis.

4. Discussion

Ischemic heart disease is a common cardiovascular disease.[23] MI has become one of the most serious clinical problems, and its incidence rate and mortality are increasing in young people.[24] During the recovery period after AMI, CFs can secrete excessive levels of ECM proteins such as collagen and fibronectin, resulting in heart failure. Therefore, alleviating activation is key to improving the prognosis of myocardial fibrosis after MI.[23] In our study, we found that circMACF1 and SMAD7 expressions was decreased and that miR-16-5p was increased in AMI patients. Overexpression of circMACF1 could inhibited the activation of TGF-β-treated CFs and suppressed migration and cell growth by mediating miR-16-5p/SMAD7 signaling.

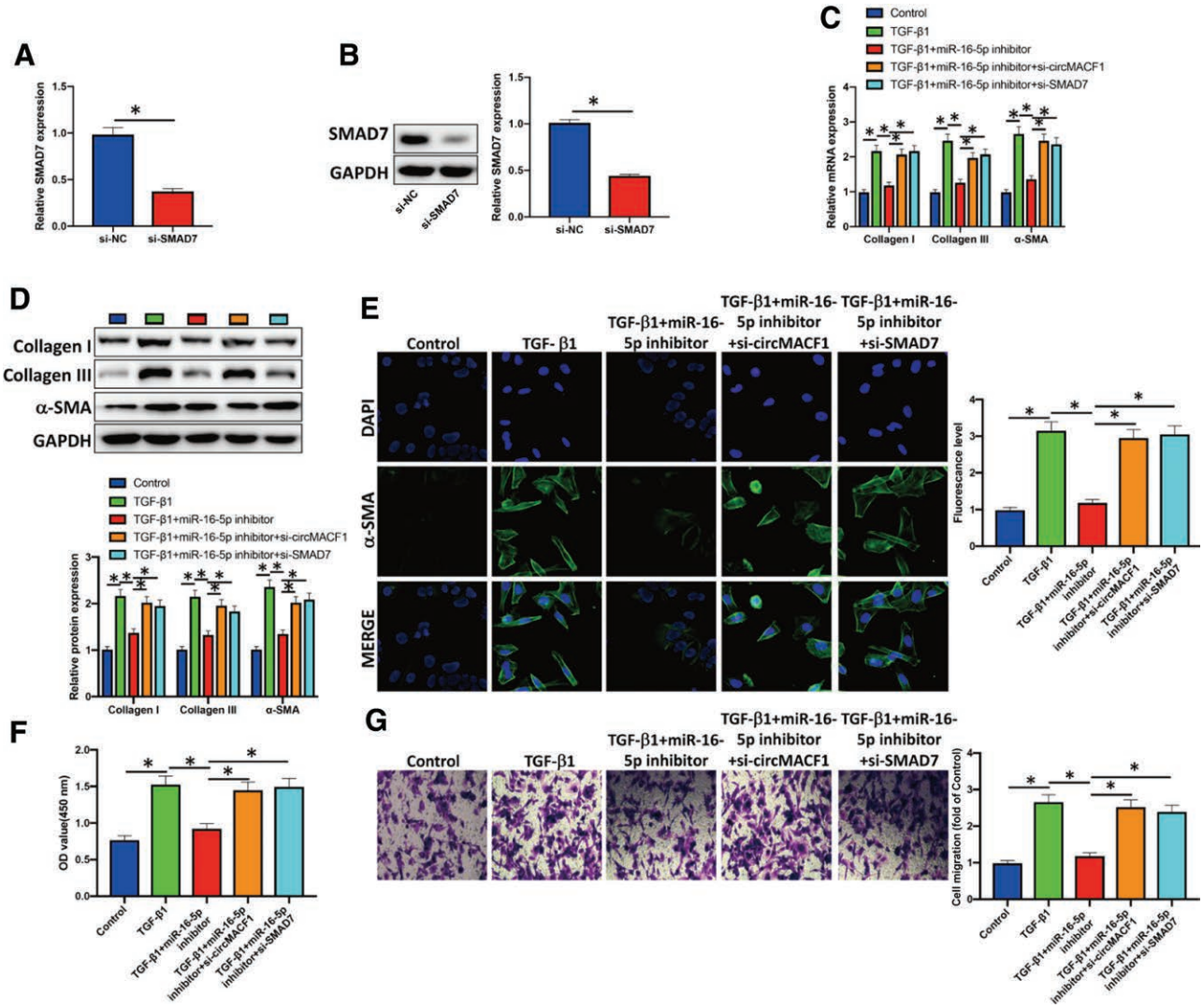

Figure 5. CircMACF1 inhibited the activation, migration and proliferation of TGF-β1-treated CFs by regulating the miR-16-5p/Smad7 axis. CFs were transfected with SMAD silencing plasmid (50 nM) for 48 hours. (A) SMAD7 levels were measured by qPCR. (B) The protein level of SMAD7 was measured by western blotting. CFs were transfected with miR-16-5p inhibitor (100 nM) and/or si-circMACF1 or si-SMAD7 (50 nM) before the treatment with TGF-β1 (10 ng/mL) for 48 hours. (C, D) Fibrosis-related protein levels were measured via qPCR and western blotting. (E) Immunofluorescence was performed to measure α-SMA expression. (F) Cell proliferation was measured through CCK-8 assay. (G) Cell migration was measured by transwell assay. All the data are presented as the mean ± standard deviation. * $P < .05$. CCK-8 = cell counting kit-8, CFs = cardiac fibroblasts, circMACF1 = circular microtubule actin crosslinking factor 1, qPCR = real time fluorescent quantitative PCR, SMAD7 = mothers against decapentaplegic homolog 7, TGF = transforming growth factor.

Noncoding RNAs, such as circRNAs or lncRNAs, participate in the pathological process of cardiovascular disease through a variety of mechanisms. Recently, circRNAs have been shown to exert a pivotal effect in the treatment and prevention of myocardial fibrosis after MI.[25] A study demonstrated that circAPAC2 could promote the cell apoptosis of cardiomyocytes in MI model rats by targeting miR-29.[26] Zhai et al[27] indicated that downregulation of circ-0060745 repressed peritoneal macrophage migration and suppressed cardiomyocyte apoptosis and inflammation. In addition, circ_LAS1L plays a protective role in myocardial fibrosis by blocking the activation of CFs and cell migration.[28] Consistent with the above argument, in the present study, circMACF1 was repressed in AMI patients and activated CFs, and circMACF1 overexpression decreased the levels of fibrosis-related proteins, and suppressed migration and cell growth.

The sponging effect of circRNAs on miRNAs is an important mechanism by which circRNAs perform their biological functions.[28] MiRNAs participate in the progression of MI-related diseases by regulating cell proliferation, differentiation and apoptosis.[23,29] A previous study displayed that overexpression of miR-223 promoted cell growth and migration, but enhanced fibrosis-related protein expression, suggesting that inhibition of miR-223 prevented against heart function failure and cardiac fibrosis.[30] Furthermore, miR-144-3p increased myocardial fibrosis after MI by promoting collagen production, cell migration and growth via regulating PTEN.[31] Our results demonstrated that miR-16-5p was increased in AMI tissues and CFs, and inhibition of miR-16-5p prevented the cell proliferation and migration of TGF-β1-treated CFs. Moreover, inhibition of miR-16-5p could suppress the activation of CFs, as evidenced by suppression of fibrosis-related protein expression. Additionally, miR-16-5p was increased by circMACF1 overexpression and enhanced by circMACF1 silencing. CircMACF1 inhibition abrogated the effect of miR-16-5p repression on CF activation, proliferation and migration.

TGF performs a pivotal function in the process of tissue fibrosis, and the SMAD protein is the main signaling protein downstream of TGF-β1.[32,33] Research has shown that miR-195 promotes the myocardial fibrosis by regulating the

TGF-β1/SMAD signaling axis.[34] It was reported that miR-20b-5p increased myocardial infarction size and myocardial collagen in rats by inhibiting SMAD7 expression through activating the TGF-β1 pathway.[35] Inhibition of miR-21 reduces the levels of Collagen I and α-SMA and the fibrosis process by mediacting SMAD7.[21] In our research, it found that SMAD7 was reduced in AMI patients and activated CFs. Moreover, inhibition of miR-16-5p markedly enhanced the SMAD7 level, and this effect was abolished by knockdown of circMACF1, indicating that circMACF1 could promote SMAD7 expression by sponging miR-16-5p. In addition, silencing SMAD7 could eliminate the regulatory role of miR-16-5p inhibition on the activation, proliferation and migration in TGF-β1-treated CFs. Taken together, these results suggest that circMACF1 may improve myocardial fibrosis after MI by suppressing CF activation via the miR-16-5p/SMAD7 axis. However, this study also has some shortcomings. For example, this study did not confirm the conclusions of the study at the level of in vivo experiments, but in the follow-up studies, we will further establish animal models and verify our conclusions.

In summary, circMACF1 reduced the activation of CFs and inhibited cell migration and proliferation by regulating SMAD7 expression by targeting miR-16-5p. It is suggested that circMACF1 could be a potential therapeutic marker in the prevention and treatment of myocardial fibrosis after AMI.

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Investigation: Aike Fei.
Methodology: Yonghong Wang.
Supervision: Liming Tan.
Validation: Yonghong Wang.
Visualization: Aike Fei.
Writing – original draft: Yonghong Wang.
Writing – review & editing: Liming Tan.

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