MicroRNA-150 Protects the Heart From Injury by Inhibiting Monocyte Accumulation in a Mouse Model of Acute Myocardial Infarction

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Background—MicroRNAs (miRs) and inflammatory monocytes participate in many cardiac pathophysiological processes including acute myocardial infarction (AMI). Recently, we observed that miR-150 is downregulated in injured mouse plasma after AMI as well as in human infarced monocytes. However, the precise functional role of miR-150 in response to AMI remains unknown.

Methods and Results—In a mouse model of AMI and in human subjects with AMI, we found that miR-150 expression was reduced in monocytes. In vitro studies showed that ectopic expression of miR-150 markedly reduced monocyte migration and proinflammatory cytokine production, whereas blockade of miR-150 had opposing effects. In vivo studies showed that overexpression of miR-150 in mice resulted in improved cardiac function, reduced myocardial infarction size, inhibition of apoptosis, and reduced inflammatory Ly-6C<sup>hi</sup> monocyte invasion levels after AMI. Wild-type mice transplanted with miR-150 null (−/−) bone marrow cells could reverse this protective effect. Mechanistic studies demonstrated that miR-150 inhibited the expression of chemokine receptor 4 (CXCR4), thereby promoting monocyte migration.

Conclusions—Our findings indicate that miR-150 acts as a critical regulator of monocyte cell migration and production of proinflammatory cytokines, leading to cardioprotective effects against AMI-induced injury. Thus, miR-150 may be a suitable target for therapeutic intervention in the setting of ischemic heart disease. (Circ Cardiovasc Genet. 2015;8:11-20. DOI: 10.1161/CIRCGENETICS.114.000598.)

Key Words: acute myocardial infarction ■ microRNAs ■ monocytes

Myocardial infarction (MI) is caused by the sudden loss of oxygen and nutrient supply to the heart, leading to cardiomyocyte death. Cardiac cell death and the inflammatory cascades that are particularly induced by ischemic injury seem to play a key role in acute injury and the postinfarction repair process.¹

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The acute inflammatory response leads to the mobilization and recruitment of innate immune cells.²,³ A few hours after the ischemic insult, neutrophils are actively recruited into the ischemic tissue and contribute to tissue inflammation and cardiovascular injury through the production of inflammatory mediators, reactive oxygen species, and various proteases.⁴,⁵ This wave of neutrophil infiltration is followed by the mobilization and recruitment of monocytes.⁶ Recent studies have shed light on the mechanisms by which monocytes are recruited to the heart as well as the life cycle of the recruited monocytes in the setting of acute MI (AMI). Specifically, these studies have suggested that Ly-6C<sup>hi</sup> and Ly-6C<sup>lo</sup> monocytes have pathogenic and protective roles, respectively, in cardiac remodeling and preservation of heart function.⁷,⁸

MicroRNAs (miRs) are small 21- to 29-nucleotide non-coding RNAs that direct proteins to repress the expression of miRNAs.⁹ MiRs have been identified as valuable therapeutic targets in a variety of diseases, including cardiovascular disease.⁹-¹¹ Recent studies have revealed that the circulating levels of miR-150 were reduced in patients with first ST-segment-elevation AMI and were associated with left ventricular (LV) remodeling.¹² In addition, knockdown of miR-150 in bone marrow–derived mononuclear cells resulted in their higher migration ability in AMI mice, influencing the repair of ischemic tissue.¹³ Although miR-150 is enriched in monocytes,¹⁴ few reports thus far have addressed the presence and functional
The aim of this study was to determine the role of miR-150 in the function of monocytes to test the hypothesis that enforced expression of miR-150 in inflammatory monocytes after MI improves infarct healing and attenuates LV remodeling.

Materials and Methods

Patient Characteristics

Between March 2013 and June 2014, 50 subjects (30 with first ST-segment–elevation AMI, 20 controls) were enrolled in our study. All subjects had no history of MI. Thirty patients were definitely diagnosed by coronary angiography. None of them received primary percutaneous coronary intervention. All controls had no coronary artery disease or angina.

Blood samples were collected at days 3 and 5 after the first AMI. The investigational protocol was approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology. Informed consent was obtained from all study participants.

Selection of Monocytes

Peripheral blood monocyte cells (PBMCs) were isolated by Ficoll-Hypaque (Pharmacia, Freiburg, Germany) density gradient separation techniques. A total of 2×10^7 cells were resuspended in 160 μL of PBS containing 40 μL of CD14 microbeads (catalog No. 130-050-201; Miltenyi Biotec, Bergisch-Gladbach, Germany). After incubation for 15 minutes at 4°C, the cells were washed and resuspended in 0.5 mL of PBS. The samples were loaded onto a MS column that was positioned in a MiniMACS magnet. Nonadherent cells were recovered, collected, and termed as CD11b+ monocytes. CD11b− monocytes were isolated from mouse PBMCs using the MACS system (catalog No. 130-096-354; Miltenyi Biotec). To keep the viability of monocytes and the stability of miRNAs, we separated CD14+ cells from the peripheral blood by MACS, although the sensitivity and purity of Fluorescence Activated Cell Sorter (FACS) sorting is slightly higher than MACS.

miR Isolation and Quantitative Reverse Transcriptase Polymerase Chain Reaction

Blood samples for miRNA analysis were collected and processed within 20 minutes of collection using 2-step centrifugation. The supernatant was transferred to RNase/DNase-free tubes and stored at −80°C. Total RNA in plasma was isolated using a MirVana PARIS kit (Invitrogen) following a modified version of the manufacturer’s instructions. For real-time polymerase chain reaction, a target-specific TaqMan hybridization probes (TaqMan miR-150 microRNA assay; Applied Biosystems) were used as a loading control to normalize expression levels for cell or plasma.

Mice

Male C57BL/6 mice, aged 8 to 10 weeks, were purchased from Beijing University (Beijing, China). MiR-150−/− mice were obtained in a C57BL/6 background and were purchased from The Jackson Laboratory. All animal studies were approved by the Animal Care and Utilization Committee of Huazhong University of Science and Technology.

MI in Mice

MI was induced by coronary ligation.14 Briefly, mice were anesthetized with ketamine (50 mg/kg) and pentobarbital sodium (50 mg/kg), orally intubated, and connected to a rodent ventilator. The chest wall was shaved, and a thoracotomy was performed in the fourth left intercostal space. The left ventricle was visualized, and the left coronary artery was permanently ligated with a monofilament nylon 8-0 suture at the site of its emergence from under the left atrium. The thoracotomy was closed with 7-0 nylon sutures, and the skin was sealed with superglue.

In Vivo Administration of AgomiR-150

AgomiRs oligonucleotides synthesized by GenePharma were deprotected, desalted, and purified by high-performance liquid chromatography. Sequences are 5'-UCUCCCAACCCGUAAGCAGUG CUGGUACAGGGGGAGAU-3' (agomiR-150). Male C57BL/6 mice (8–10 weeks old) received either agomiR-150 or mutant agomiR-150 through 2 consecutive daily injections via the tail vein once a week for 2 weeks (2×20 mg/kg body weight). MI was performed on the third day after the first injection.

Bone Marrow Reconstitution

Briefly, wild-type (WT) mice received 12 Gray split into 2 doses. Recipient mice were reconstituted with 2×10^7 miR-150−/− bone marrow cells. Similarly, WT mice were reconstituted with miR-150−/− bone marrow and WT mice were reconstituted with WT bone marrow (control). After 6 weeks, the mice were subjected to AMI.

Echocardiographic and Hemodynamic Analysis of Cardiac Function

A Vevo 2100 high-resolution microimaging system with a 30-MHz transducer was used (Visualsonic, Toronto, Ontario, Canada). Mice were anesthetized with 1.5% isoflurane, and 2-dimensional echocardiographic views of the midventricular short axis and parasternal long axes were obtained. LV fractional shortening and LV ejection fraction were calculated from digital images using a standard formula as previously described.6–7 Echocardiographic acquisition and analysis were performed by a technician who was blinded to the treatment groups.

Immunohistochemistry on Mouse Hearts

For morphometric analysis, hearts were harvested from mice at each time point. The hearts were fixed overnight in 10% buffered formalin. The tissue was paraffin embedded and sectioned at 4 μm; hematoxylin and eosin/Masson’s trichrome staining was performed using routine techniques. To measure the infarct size at 2 weeks after AMI, sections from 4 equally distributed levels were Masson’s trichrome stained. Scar size was calculated as the percentage of the LV circumference or total scar area divided by total LV area, and was summed from 4 longitudinal sections per heart. To analyze infarcted areas for the vessel content, sections were stained with CD31 antibody. To quantify apoptotic cardiomyocytes, mouse hearts were removed 3 days after coronary artery ligation, fixed with 0.5% paraformaldehyde in 5% sucrose, routinely frozen embedded in optimal cutting temperature (OCT), and processed for sectioning and staining with TUNEL according to the manufacturer’s directions. Five randomly chosen fields from each dish were counted for to determine the percentage of apoptotic nuclei. Frozen heart sections were also stained with CD11b.

Flow Cytometry

Mice were euthanized on day 7 after AMI (n=5 mice). Peripheral blood was drawn via cardiac puncture with citrate solution, and anticoagulant and mononuclear cells were purified by density centrifugation as previously described.15 Spleens were removed, triturated in Hanks’ balanced salt solution at 4°C with the end of a 3-mL syringe and filtered through nylon mesh (BD Biosciences). The cell suspension was centrifuged at 300g for 10 minutes at 4°C and purified by density centrifugation. Infarct tissues were harvested; minced with ice scissors, placed into a cocktail of collagenase I, collagenase XI, DNase I, and hyaluronidase (Sigma-Aldrich), and shaken at 37°C for 1 hour, as previously described.15 Cells were then triturated through nylon mesh and centrifuged (15 minutes, 500g, 4°C). Cell suspensions were incubated in a cocktail of
of monoclonal antibodies against CD11b-PerCP and Ly6C-APC (BD Biosciences).

**Cell Culture and miR Transfection**

THP-1 cells, a human monocytic leukemia cell line, were purchased from the American Type Culture Collection. Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and l-glutamine. miR (agomiR-150 and negative controls [NCs]) and miR inhibitors (antagomiR-150 and NCs) were synthesized by Shanghai GenePharma Co (Shanghai, China). THP-1 cells were seeded into 6-well plates for 12 hours. THP-1 cells were simultaneously stimulated with phorbol 12-myristate 13-acetate at a final concentration of 200 ng/mL after seeding into the culture plate. The cells were put in fresh medium (1640+10% fetal bovine serum) and transfected with agomiR-150 (5′-UCUCCGAACGU GUAACUGGUACAAGGGUUGGAGAUU-3′)/agomir NC (5′-UUCUCCGAACGU GUAACUGGUACAAGGGUUGGAGAUU-3′) or antagomiR-150 (5′-UCUCCGAACGU GUAACUGGUACAAGGGUUGGAGAUU-3′)/antagomir NC (5′-UCUCCGAACGU GUAACUGGUACAAGGGUUGGAGAUU-3′) at a concentration of 50 pmol/well using Lipofectaime 2000 (Invitrogen, USA), according to the manufacturer’s instructions. After transfection for 6 to 12 hours, the medium was replaced with fresh medium containing 2 mg/mL lipopolysaccharide. The cell medium and lysis samples were then collected according to the aforementioned protocol after lipopolysaccharide induction for 24 hours.

**Luciferase Reporter Assays**

For luciferase reporter experiments, a CXCR4 3′-UTR segment (5′-GGTATAGAAATGCTGGTTTTTTCATTTTTCAGGAGTG GGTGATTTTGACACT-3′) and its respective mutant (5′-GGTATAGAAATGCTGGTTTTTTCATTTTTCAGGAGTG GGTGATTTTGACACT-3′) were amplified and inserted into the psiCHECK-2 Vector luciferase miRNA expression reporter vector (GenePharma). Luciferase reporter assays were performed in HEK293T cells. HEK293T cells were seeded into 96-well plates and transfected with either 100 ng reporter constructs or corresponding mutants and 10 pmol agomiR-150 or control using Lipotectamine LTX and Plus Reagent (Invitrogen). Luciferase activity was measured using the Dual-Glo Luciferase Reporter Assay system (Promega). All assays were performed in triplicate.

**Migration**

Migration experiments were performed in transwell cell culture chambers as previously described. Transfected THP-1 cells were centrifuged, washed in PBS, centrifuged, and resuspended in RPMI 1640 medium containing 0.2% fetal bovine serum; 5×10^4 THP-1 cells were placed on a gelatin-coated polycarbonate membrane with 8-mm pores and incubated at 37°C for 1 hour, allowing the cells to attach. Migration was induced by the addition of RPMI 1640 medium containing 10% fetal bovine serum to the lower compartment. After 4 hours, nonmigrating cells were removed with a cotton tip and the membranes were fixed and stained with Giemsa stain set to identify migrated cells. The number of migrated cells was determined per ×200 high-power field. Experiments were performed in duplicate and were repeated ≥3×.
Western Blot Analysis

Cell extracts were separated by SDS-PAGE (12%) and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat dry milk for 1 hour and left to react overnight with rabbit polyclonal anti-CXCR4 antibody (1:1000; Sigma). After rinsing, the membranes were soaked in blocking buffer with antirabbit secondary antibody (1:1000) for 1 hour. After rinsing again, the membranes were used to visualize the blots by the chemiluminescence method (KPL, USA).

Enzyme-Linked Immunosorbent Assay

For cytokine production measurements, THP-1 cell supernatants were assayed for cytokine levels using a human cytokine quantibody array (RayBiotech) according to the manufacturer’s instructions.

Statistical Analysis

The quantitative data were evaluated for a normal distribution using the Shapiro–Wilk test. Normally distributed continuous variables were presented as mean±SD.

Continuous variables that were not normally distributed were presented as medians. Baseline characteristics were assessed using t tests and Spearman rank correlation coefficient for continuous variables and χ² tests for categorical variables. Statistical analysis was performed by 1-way ANOVA. Whenever a significant effect was obtained with ANOVA, multiple-comparison tests between the groups with the Student–Newman–Keuls procedure were performed. Two groups were compared using paired, 2-tailed t test (SPSS 20.0 statistical program or Graphpad). Nonparametric tests were used to compare the data of the small sample sizes in Figures 1–5. P<0.05 was considered statistically significant.

Results

MiR-150 Is Downregulated in Monocytes After AMI

Recent studies have reported that lower circulating concentrations of miR-150 are associated with LV remodeling. Plasma samples from patients with AMI (n=30) and healthy controls (n=20) were collected to analyze the expression of miR-150 (Table). Compared with the healthy group, the levels of miR-150 were reduced by 2.1±0.5-fold in plasma from patients with AMI at day 3 and were reduced even further by 4.5±0.4-fold at day 5 (Figure 1A in the Data Supplement). The miR-150 levels were reduced significantly in plasma from patients with AMI at days 3 and 5 compared with the healthy group (P<0.05 or P<0.01). MiR-150 is abundantly expressed in monocytes, which are a critical source of proinflammatory cytokines in AMI. To determine miR-150 expression in monocytes, we separated CD14+ monocytes from the peripheral blood of AMI patients and healthy controls using the CD14 MicroBead...
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kit (CD14− and CD14+ purity, 13.18% and 88.35%; Figure IC and ID in the Data Supplement). We found that the expression of miR-150 was rather low in monocytes (Figure 6A). However, the expression of miR-150 was not significantly different in CD14- cells of PBMCs between AMI patients and healthy controls (Figure 6B).

To further determine the source of miR-150 dysregulation, we measured miR-150 levels in mice on days 3 and 5 after coronary ligation (n=5 per group). The expression levels of miR-150 in plasma and CD115+ monocytes, which were separated from PBMCs, were both significantly reduced (Figure IB in the Data Supplement and Figure 6C). Consistent with previous studies, the expression of miR-150 was not significantly different between CD115- PBMCs in mice after AMI and WT controls (Figure 6D). Together, these data demonstrate that miR-150 is downregulated in monocytes during AMI in both mice and humans.

**MiR-150 Regulates Migration and Production of Proinflammatory Cytokines in Human Monocytes**

Dysregulation of miR-150 expression in AMI monocytes suggested that it could also play a role in monocyte function. To test this possibility, human THP-1 monocytes were transfected with control or agomiR-150. Overexpression of miR-150 inhibited the migration of THP-1 monocytes, resulting in a 3.7±0.5-fold inhibition in THP-1 cells compared with the control (Figure 1A, upper). Furthermore, miR-150 overexpression decreased lipopolysaccharide-induced proinflammatory cytokine production compared with the control (Figure 1B). Next, THP-1 cells were transfected with either a control or an miR-150 inhibitor (antagomirs). Downregulation of miR-150 significantly stimulated the migration of THP-1 cells with a 6.3±0.8-fold induction (Figure 1A, lower). Finally, to confirm that miR-150 could be involved in the post-transcriptional control of the inflammatory pathways in monocytes, THP-1 cells were reactivated by lipopolysaccharide stimulation after transfection with either the control or the miR-150 antagomir. Interestingly, downregulation of miR-150 triggered a significant increase in interleukin (IL)-1β and IL-6 production (Figure 1C). Altogether, these data indicate that aberrant expression of miR-150 could cause monocytes to exhibit proinflammatory and migratory phenotypes.

**Overexpression of miR-150 Improved Postischemic Recovery of Cardiac Function**

To assess the effect of miR-150 during AMI, mice were intravenously injected with the indicated doses of agomiR-150 or control through 2 consecutive daily injections before myocardial ischemia. Overexpression of miR-150 in monocytes but not cardiomyocytes (Figure II in the Data Supplement) excluded the possibility that miR-150 regulated the function of cardiomyocytes. Cardiac function was assessed 2 weeks after reperfusion by echocardiography and compared with controls. Overexpression of miR-150 improved postischemic recovery of cardiac function, as evidenced by increased heart function, reduced infarct size, and reduced cell death (Figure 3A-E). Additionally, neovascularization was reduced in agomiR-150–injected mice compared with controls (Figure 3E).

Figure 3. MicroRNA (miR)-150 overexpression attenuated acute myocardial infarction (AMI)–induced myocardial damage. A, Representative M-mode echocardiography image of a heart at 14 days after AMI. B, MiR-150 overexpression showed improved cardiac function at 14 days after AMI (n=5 for wild-type [WT] negative control [NC], n=8 for agomiR-150 mice; *P<0.05). C, The infarct area was reduced in agomiR-150–injected mice compared with the controls at 14 days after AMI (n=4 per group, **P<0.01). D, TUNEL analyses of control NC or agomiR-150–treated infarcted heart sections. *P<0.05. E, Neovascularization was reduced in agomiR-150–injected mice compared with the controls at 14 days after AMI. n=4 mice per group and n=4 high-power field per mouse. *P<0.05. CO indicates cardiac output; EF, ejection fraction; FS, fractional shortening; LV, left ventricular; LVd diameter, diastolic LV diameter; LVs diameter, systolic LV diameter; and TUNEL, terminal deoxynucleotidyl transerase dUTP nick-end labeling.
after AMI using high-resolution echocardiography (Figure 2A). MiR-150–treated mice exhibited significantly better cardiac function than the control groups, as determined by the systolic and diastolic LV diameter and ejection fraction (Figure 2B). These data reveal that increased levels of miR-150 (≈3–8-fold) are associated with improved functional recovery of the heart after AMI.

Overexpression of miR-150 Reduces AMI-Induced Cardiac Injury

To assess the cardioprotective effects of miR-150, we measured the infarct size in mice after AMI. Fourteen days after AMI, the infarct size was reduced in miR-150–treated mice by 55% when compared with the control groups (Figure 2C). It is well appreciated that maintaining adequate numbers of cardiomyocytes is critical to the preservation of cardiac structural integrity and function after AMI.23–25 Thus, next we analyzed the extent of apoptotic cell death after AMI. The number of TUNEL-positive nuclei was significantly reduced after AMI in the miR-150–treated mice compared with the control groups (Figure 2D). However, neovascularization was impaired in miR-150–treated mice compared with WT mice after AMI, as evidenced by the reduction in the formation of CD31+ blood vessels in the infarcted myocardium (Figure 2E). These results indicate that miR-150 protects the myocardium through the inhibition of apoptosis as opposed to neovascularization.

Ly-6C<sup>hi</sup> monocytes are recruited early to inflammatory environments and thought to be proinflammatory; in addition, they express tumor necrosis factor-α, IL-1β, myeloperoxidase, and IL-6.22 Inflammation exacerbates the injury by harming the myocytes that survive the ischemic period. Next, we analyzed the mobilization and recruitment of inflammatory monocytes after AMI. Indeed, flow cytometric analysis showed reduced numbers of inflammatory Ly-6C<sup>hi</sup> monocytes (P<0.05) in infarcts, peripheral blood, and spleens of mice treated with miR-150 (n=5 per group; Figure 3B). These findings were validated by immunostaining studies on hearts on day 7 after AMI. Mice treated with miR-150 exhibited fewer CD11b<sup>+</sup> myeloid cells compared with controls (Figure 3A). Moreover, quantitative reverse transcriptase polymerase chain reaction of infarcted myocardium samples revealed that the overexpression of miR-150 reduced the expression of inflammatory genes, including tumor necrosis factor-α and IL-1β. IL-10 gene expression was also greater in miR-150–treated hearts (Figure 3C). These results are consistent with our previous findings that suggest a cardioprotective role for miR-150 after AMI.

CXCR4 Are Targets of miR-150

To further validate whether miR-150 directly recognizes 3′-UTRs of CXCR4, we generated luciferase reporter constructs harboring a segment of CXCR4 3′-UTR (Figure 4A). Their respective mutant constructs contained a mutated seed sequence. These luciferase reporter constructs were cotransfected into HEK293 cells with either miR-150 or miR control. Forty-eight hours after transfection, we conducted luciferase activity assays and observed that miR-150 significantly repressed luciferase activity, whereas no inhibitory effect was
observed with their corresponding mutated constructs (Figure 4B). We thus identified CXCR4 as direct targets of miR-150. In addition, overexpression of miR-150 in THP-1 cells reduced protein levels of CXCR4 ($P<0.05$; Figure 4C), but not its mRNA levels (Figure IIIA in the Data Supplement). Furthermore, the CXCR4 levels in CD115+ monocytes, which were separated from miR-150–treated mice, were also reduced as compared with WT controls (Figure IIIB in the Data Supplement).

Knockdown of miR-150 Renders the Heart More Sensitive to AMI Injury

MiR-150 null (−/−) mice were obtained to recapitulate the in vivo setting where miR-150 is downregulated in the monocytes after AMI. To distinguish the influence of the downregulated miR-150 expression in cardiomyocytes, bone marrow chimera experiments were performed after lethal irradiation. Two weeks after AMI, WT mice transplanted with miR-150−/− bone marrow exhibited significantly depressed cardiac function compared with the control groups, as evidenced by the

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**Table. Patient Characteristics**

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<td><strong>cTnI, ng/L</strong></td>
<td>193 (50–753)</td>
<td>4 (3–9)</td>
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Values are mean±SD, n (%), or median (interquartile range). AMI indicates acute myocardial infarction; CK-MB, creatine kinase-myocardial band; and cTnI, cardiac troponin I.
decreased systolic and diastolic LV diameter and ejection fraction (Figure 5A). In addition, the infarcted areas were significantly increased in WT mice transplanted with miR-150−/− bone marrow (Figure 5B). Similarly, the number of TUNEL-positive nuclei after induction of AMI was significantly increased in the hearts of WT mice transplanted with miR-150−/− bone marrow compared with the control groups (Figure 5D). Conversely, the increase of neovascularization was more pronounced in mice transplanted with miR-150−/− bone marrow (Figure 5C).

Interestingly, AMI-induced infiltration of the peripheral blood and spleens with inflammatory Ly-6Chigh monocytes was drastically increased in WT mice transplanted with miR-150−/− bone marrow at day 7 after AMI (Figure 5E). Thus, the initial inflammatory response differed markedly in WT/miR-150−/− bone marrow (Figure 5C).


data indicate that the reduction of endogenous levels of miR-150 in monocytes sensitizes the heart to AMI-induced injury.

Discussion

Our data demonstrate that the expression of miR-150 in peripheral monocytes was reduced during AMI in both mice and humans. Compared with WT mice, mice with upregulated levels of miR-150 in monocytes revealed smaller and stable AMI scars because of attenuation of the acute inflammatory recruitment of Ly-6C<sup>high</sup> monocytes and better adaptation of cardiomyocytes to hypoxic stress. Given that inflammatory monocytes have crucial nonredundant roles in the pathogenic response to acute ischemic injury, our data provide important insights into the endogenous function of miR-150 after AMI.

Although previous studies have shown that miR-150 expression is reduced in the plasma of AMI patients, the mechanism is still not understood. Our study demonstrated downregulation of miR-150 in monocytes from human patients and a mouse model of AMI. Based on the observation that miR-150 is expressed and secreted by monocytes, our study suggests that the downregulation of miR-150 in the plasma may be because of the loss of miR-150 in monocytes.

To elucidate the functional consequences of dysregulation of miR-150 in monocytes, we exploited a mouse model of AMI. The accumulation of leukocytes occurs during the first minutes to hours after AMI. Surprisingly, monocyte recruitment may outpace neutrophils soon after coronary ligation. Blood monocytosis by itself causes excessive monocyte recruitment in infarcts, which results in impaired infarct healing and accelerated LV dilation. The inflammatory Ly-6C<sup>high</sup> monocyte subset is recruited during the first few days after AMI via the chemokine monocyte chemotactic protein-1, CCR2, and CXCR4. Ly-6C<sup>high</sup> monocytes have a high payload of inflammatory cytokines, such as tumor necrosis factor-α and proteases. Therefore, we injected agomiR-150 to mice before coronary ligation, to make sure that accumulated monocytes in injured myocardium had expressed high miR-150. We found that a low-dose treatment of agomiR-150 (overexpression of miR-150 in monocytes and not cardiomyocytes)
reversed the number of recruited Ly-6C<sup>high</sup> monocytes and lowered their numbers in the blood, spleen, and infarcted tissue of AMI mice compared with WT mice. Of note, an association between increased blood monocyte levels and LV remodeling has been described recently in patients after AMI. Recently, a study reported that low circulating miR-150 levels are associated with LV remodeling after first ST-segment-elevation MI. Our study strongly suggests that downregulation of miR-150 in circulation accelerated the mobilization of Ly-6C<sup>high</sup> monocytes and the proinflammatory properties of miR-150<sup>−/−</sup>, which may deteriorate immune activity in patients with acute coronary syndromes, partly contributing to their prognosis.

Reduced inflammatory Ly-6C<sup>high</sup> monocyte infiltration itself may substantially affect myocardial injury and reduce infarct size. In line with these results, the infarct size was significantly reduced in miR-150<sup>+</sup> mice. Downregulation of miR-150 resulted in the opposite effect.

The role of miR-150 in the heart is poorly characterized. A prominent characteristic of miR-150 is that it potentially regulates both apoptosis and inflammation. We supposed that CXCR4, which contributes to inflammatory/progenitor cell recruitment, as a major miR-150 target. In the present study, CXCR4 levels were reduced in miR-150<sup>−/−</sup> monocytes in vivo and ex vivo. Similarly, a recent study revealed that CXCR4<sup>−/−</sup> mice displayed diminished levels of Gr1<sup>high</sup> monocytes, smaller and stable AMI, and impaired myocardial neovascularization after AMI. This is keeping in line with mice, which overexpressed miR-150 after AMI, implying that CXCR4 has an important role in the physiological response of miR-150 to acute ischemic injury.

In addition, suppression of overwhelming inflammation by miR-150 may limit the amount of cell loss caused by AMI. Overexpression of miR-150 in peripheral cells consistently reduced cardiomyocyte apoptosis. Furthermore, the antiapoptotic effect of miR-150 has been reported in A549 cancer cells and likely is mediated via the upregulation of P53. In our study, overexpression of miR-150 in peripheral cells consistently inhibited apoptosis of cardiomyocytes. However, the miR-150 level of cardiomyocytes was not altered in the mouse model. Hence, we speculate that the suppression of overwhelming inflammation by miR-150 may limit the cell loss caused by AMI. Furthermore, we found that miR-150 inhibited recruitment of proinflammatory Ly-6C<sup>high</sup> monocytes, which promoted the digestion of infarcted tissues and removal of necrotic debris. This may also contribute to the reduced cardiomyocyte apoptosis. However, the mechanisms by which miR-150 affects myocardial apoptosis are unknown and deserve further research.

Recently, Rolland-Turner et al. found that adenosine decreased the expression of miR-150 and increased expression of CXCR4 in endothelial progenitor cells, thereby enhancing the capacity of EPC to revascularize the ischemic heart. The result was consistent with our finding that overexpression of miR-150 reduced AMI-induced cardiac injury with impaired neovascularization.

In summary, these studies established the critical role for miR-150 in monocytes that lead to tissue damage after AMI. We did not exclude the possibility that aberrant miR-150 expression in circulation affects the function of neutrophils, B cells, natural killer cells, and T cells after AMI. Indeed, these cells enter the sites of injury and release inflammatory mediators, facilitating the mobilization of monocytes from the bone marrow and thereby enhancing entry of monocytes into sites of injury. Ultimately, monocytes, which accumulate quickly so as to engulf and digest dead or dying tissues, play an important and fine-tuned role in the process of cardiac repair. In addition, miR-150 is abundantly expressed in monocytes. In the peripheral blood plasma of MI patients, change in MiR-150 expression was in line with its alternation in peripheral monocytes. Therefore, we targeted the possibility that miR-150 plays a role in the function of monocytes. Although overall elevation of circulating miR-150 may influence the function of neutrophils, B cells, natural killer cells, and T cells, it did promote postischemic recovery of cardiac function in vivo. Importantly, our studies discovered that miR-150 played a critical role in the function of monocytes both in vivo and in vitro. Taking these findings together, we are led to postulate that change in peripheral miR-150 might affect the post-MI cardiac functions by changing the function of monocytes.

In conclusion, we demonstrated that the protective effects of miR-150 in AMI is mediated, at least in part, via activation of CXCR4, and that miR-150 treatment has a profound impact on the innate immune response after AMI. The present observations may provide a basis for novel therapeutic strategies aimed at enhancing cardiac function in the ischemic myocardium.

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Disclosures

None.

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CLINICAL PERSPECTIVE

MicroRNAs (miRs), a new class of non–protein-coding small RNAs, have emerged as regulators that control the expression of hundreds of proteins. As a consequence, they have been identified as valuable therapeutic targets in a variety of diseases, including cardiovascular disease. Although previous studies have revealed that the circulating levels of miR-150 were reduced in patients with acute myocardial infarction and were associated with left ventricular remodeling, the mechanism is still not understood. In the present study, we discovered that miR-150 expression was reduced in monocytes from human patients and a mouse model of acute myocardial infarction and ectopic expression of miR-150 markedly reduced monocyte migration and proinflammatory cytokine production. In vitro, suggesting that the downregulation of miR-150 in the plasma may be because of the loss of miR-150 in monocytes. Furthermore, increased levels in peripheral mature miR-150 rendered cardioprotection against myocardial infarction–induced injury, whereas blockade of miR-150 had opposing effects. Importantly, we identified that miR-150–targeted CXCR4 in monocytes, consequently, improved their migration capacity and significantly reduced the production of proinflammatory cytokines. These data suggest that downregulation of miR-150 observed in human peripheral monocytes may be causally involved in the progression of myocardial infarction, at least in part.
MicroRNA-150 Protects the Heart From Injury by Inhibiting Monocyte Accumulation in a Mouse Model of Acute Myocardial Infarction
Zheng Liu, Ping Ye, Sihua Wang, Jie Wu, Yuan Sun, Anchen Zhang, Linyun Ren, Chao Cheng, Xiaofan Huang, Ke Wang, Peng Deng, Chuangyan Wu, Zhang Yue and Jiahong Xia

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**Supplementary Figure 1.** (A) The miR-150 levels were reduced in plasma from patients (n=30) with AMI at day 3 and day 5 compared to the healthy group (n=20). *P*<0.05, MI 3d vs control; **P*<0.01, MI 5d vs control. (B) The miR-150 levels in plasma from AMI mice (n=5) and control WT mice (n=5). *P*<0.05, MI 3d vs control; **P*<0.01, MI 5d vs control. CD14+ cells were isolated from PBMCs of AMI patients using the CD14 MicroBead kit. CD14+ Cells (C) and CD14- cells (D) were fluorescently stained with CD14-FITC.
Supplementary Figure 2. C57BL/6 male mice (8–10 weeks old) were injected with either a low dose of agomiR-150 or mutant agomiR-150. The bar graph shows that miR-150 is upregulated in monocyte cells but not in cardiomyocytes; n=4 per group; *** P<0.001 vs. the control.
Supplementary Figure 3. (A) The mRNA levels of CXCR4 in THP-1 cells transfected with either a control or agomiR-150. (B) CXCR4 protein expression was significantly reduced in isolated CD115+ monocytes from miR-150-treated mice. (C) CXCR4 protein expression in siRNA-CXCR4 transfected THP-1 cells, as compared to controls. * $P<0.05$. 