miR-216a exacerbates TGF-β-induced myofibroblast transdifferentiation via PTEN/AKT signaling

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Received November 3, 2018; Accepted April 9, 2019

DOI: 10.3892/mmr.2019.10200

Abstract. Myofibroblast transdifferentiation is an important feature of cardiac fibrosis. Previous studies have indicated that microRNA-216a (miR-216a) is upregulated in response to transforming growth factor-β (TGF-β) in kidney cells and can activate Smad3; however, its role in myofibroblast transdifferentiation remains unclear. The present study aimed to investigate the role of miR-216a in TGF-β-induced myofibroblast transdifferentiation, and to determine the underlying mechanisms. Adult mouse cardiac fibroblasts were treated with TGF-β to induce myofibroblast transdifferentiation. An antagonir and agomir of miR-216a were used to inhibit or overexpress miR-216a in cardiac fibroblasts, respectively. Myofibroblast transdifferentiation was evaluated based on the levels of fibrotic markers and α-smooth muscle actin expression. The miR-216a antagonir attenuated, whereas the miR-216a agomir promoted TGF-β-induced myofibroblast transdifferentiation. Mechanistically, miR-216a accelerated myofibroblast transdifferentiation via the AKT/glycogen synthase kinase 3β signaling pathway, independent of the canonical Smad3 pathway. In addition, it was observed that miR-216a activated AKT via the downregulation of PTEN. In conclusion, miR-216a was involved in the regulation of TGF-β-induced myofibroblast transdifferentiation, suggesting that targeting miR-216a may aid in developing effective interventions for the treatment of cardiac fibrosis.

Introduction

Cardiac fibrosis is a common pathologic component of various cardiovascular disorders, defined as the excessive deposition of extracellular matrix (ECM) and the disturbance of myocardial stiffness, which subsequently results in systolic and/or diastolic dysfunction of the heart (1). Increased accumulation of myocardial ECM also impairs the electrical conduction system and contributes to arrhythmogenesis (2,3). Activation and transdifferentiation of cardiac fibroblasts to myofibroblasts is a crucial event in cardiac fibrosis, and is responsible for the excessive synthesis of ECM (1). Therefore, improved understanding of the pathogenesis of myofibroblast transdifferentiation and the identification of novel therapeutic targets may be of great therapeutic interest for the treatment of cardiac fibrosis.

Transforming growth factor-β (TGF-β) is the most widely known fibrogenic growth factor associated with cardiac fibrosis, and promotes the transdifferentiation of cardiac fibroblasts to myofibroblasts (4,5). In response to cardiovascular insults, bioactive TGF-β is induced and released from latent stores, subsequently binding to TGF-β receptors, resulting in the activation of the canonical Smad-dependent signaling pathway and the induction of a profibrotic gene program (6). In addition, TGF-β can stimulate myofibroblast transdifferentiation and promote ECM synthesis via non-canonical pathways, including mitogen-activated protein kinase (MAPK) and protein kinase B (AKT) (7,8). These non-canonical pathways coordinate with the Smad-dependent canonical pathway to induce cardiac fibrosis (9). Furthermore, negative regulators of AKT or p38 have been reported to inhibit myofibroblast transdifferentiation and protect against cardiac fibrosis (8,10). Thus, targeting TGF-β signaling, via canonical or non-canonical pathways, may aid in developing efficacious interventions against fibrosis.

MicroRNAs (miRNAs) are a class of small noncoding RNA molecules that function as negative regulators of gene expression by binding to the 3'-untranslated region (UTR) of target mRNAs (11,12). Emerging evidence suggests that miRNAs regulate the expression of key genes involved in fibrotic diseases, particularly cardiac fibrosis (13,14). Previous studies have demonstrated that miR-133a is downregulated in transverse aortic constriction or isoproterenol-induced fibrotic hearts, and that miR-133a overexpression can reduce collagen deposition and improve cardiac dysfunction (15). Pan et al (16) observed that forced expression of miR-101a/b suppressed the proliferation and collagen production in rat neonatal cardiac fibroblasts. Additionally, results from Nagpal et al (17)
demonstrated that mir-125b was important for the induction of cardiac fibrosis, and that the inhibition of mir-125b may represent a novel therapeutic approach for the treatment of cardiac fibrosis. These studies indicated a central role for miRNAs in cardiac fibrosis.

miRNA-216a lies in the second intron of a noncoding RNA (RP23-298H6.1-001) located on the mouse chromosome 11 (18). The majority of previous studies into mir-216a have focused on tumors, identifying mir-216a as a potential biomarker for certain types of cancer (19,20). Xia et al (20) reported that mir-216a contributed to hepatocarcinogenesis and tumor recurrence in hepatocellular carcinoma. Recent studies, however, have suggested that the functions of mir-216a extend beyond the regulation of tumors, and that it serves important roles in other pathophysiological processes. For example, Yang et al (21,22) reported that mir-216a promotes endothelial senescence and inflammation, and M1 macrophage polarization via Smad3. Additionally, it was observed that mir-216a levels were increased in mouse renal mesangial cells following stimulation with TGF-β (23). The present study hypothesized that mir-216a may be involved in the pathogenesis of myofibroblast transdifferentiation and cardiac fibrosis.

Materials and methods

Reagents. TGF-β (cat. no. ab50036) was purchased from Abcam. AKT inhibitor MK2206 (cat. no. HY-10358) was purchased from MedChemExpress LLC. The antagonist (5'-CAGCGUGGAGAUA-3') and the agonir (5'-UAUCUGACUGUGAUA-3') of mir-216a, their negative controls (antagonir control, cat. no. miR3N0000001-4-5; agonir control, cat. no. miR4N0000001-4-5), small interfering (si)RNA against PTEN (siPten; 5'-TTCCGGAC TGAACATTGGA-3') and negative control siRNA (cat. no. siN0000001-1-10) were generated by Guangzhou Ribibo Co., Ltd. Alexa Fluor® 488-anti-rabbit immunoglobulin G (IgG) secondary antibody (1:200; cat. no. A11008) for immunofluorescence detection was obtained from Pierce (Thermo Fisher Scientific, Inc.). The anti-TGF-β receptor II (T G F B R 2 ; 1 : 1 , 0 0 0 ; c a t . n o . 2 1 1 8 ) was purchased from Cell Signaling Technology, LI-COR Biosciences) in a blinded manner, and target proteins were detected using a BulgeLoop™ miRNA RT-qPCR System (Guangzhou RiboBio Co., Ltd.). The thermocycling conditions were as follows: 95˚C for 10, then 40 cycles of 95˚C for 2 sec, 45-55%; 12 h light/dark cycle) for 1 week prior to commencing the study. Adult mouse cardiac fibroblasts were isolated as previously described (25). In brief, left ventricles were harvested and digested in 0.125% trypsin and collagenase. The culture was then collected and suspended in DMEM/F12 medium with 10% fetal bovine serum (HyClone; GE Healthcare Life Sciences) medium with 10% fetal bovine serum (HyClone; GE Healthcare Life Sciences) at 37˚C for 90 min. The adherent fibroblasts were prepared for the subsequent experiments following synchronization for 12 h. The mir-216a antagonist and agonir, and their negative controls were all diluted with DMEM/F12 medium and then were mixed with Lipopectamine RNaiMAX reagent (Invitrogen; Thermo Fisher Scientific, Inc.) for 20 min at room temperature. Then, when the cells had grown to 70-80% confluency, they were incubated with the mixture at a final concentration of 50 nM at 37˚C for 24 h, followed with TGF-β stimulation for an additional 24 h. To inhibit AKT activity, cardiac fibroblasts were pretreated with MK2206 (1 μM) for 24 h (26). PTEN knockdown was performed using siPten, and the efficiency of the knockdown was assessed via western blotting. Briefly, the siPten and its negative control were diluted with DMEM/F12 medium and then mixed with Lipopectamine RNaiMAX reagent for 20 min at room temperature. Then, the cells (at 40-50% confluency) were incubated with the mixture at a final concentration of 50 nM at 37˚C for 4 h, followed by mir-216a antagonir transfection for 24 h and TGF-β stimulation for an additional 24 h as aforementioned.

Cell culture and treatments. All of the animal experimental protocols were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (approval no. 20171003) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (24). A total of 40 male C57BL/6 mice (age, 8-10 weeks; body weight, 23-28 g) were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences. The animals were allowed free access to food/water in a specific pathogen-free, environmentally controlled barrier conditions (temperature, 20-25˚C; humidity, 45-55%; 12-h light/dark cycle) for 1 week prior to commencing the study. Adult mouse cardiac fibroblasts were isolated as previously described (25). In brief, left ventricles were harvested and digested in 0.125% trypsin and collagenase. The culture was then collected and suspended in DMEM/F12 (HyClone; GE Healthcare Life Sciences) medium with 10% fetal bovine serum (HyClone; GE Healthcare Life Sciences) at 37˚C for 90 min. The adherent fibroblasts were prepared for the subsequent experiments following synchronization for 12 h. The mir-216a antagonist and agonir, and their negative controls were all diluted with DMEM/F12 medium and then were mixed with Lipopectamine RNaiMAX reagent (Invitrogen; Thermo Fisher Scientific, Inc.) for 20 min at room temperature. Then, when the cells had grown to 70-80% confluency, they were incubated with the mixture at a final concentration of 50 nM at 37˚C for 24 h, followed with TGF-β stimulation for an additional 24 h. To inhibit AKT activity, cardiac fibroblasts were pretreated with MK2206 (1 μM) for 24 h (26). PTEN knockdown was performed using siPten, and the efficiency of the knockdown was assessed via western blotting. Briefly, the siPten and its negative control were diluted with DMEM/F12 medium and then mixed with Lipopectamine RNaiMAX reagent for 20 min at room temperature. Then, the cells (at 40-50% confluency) were incubated with the mixture at a final concentration of 50 nM at 37˚C for 4 h, followed by mir-216a antagonir transfection for 24 h and TGF-β stimulation for an additional 24 h as aforementioned.

Western blotting. Western blotting was performed as previously described (27,28). Briefly, cultured cardiac fibroblasts were lysed in RIPA lysis buffer (50 mM Tris-HCl, 0.5% NP-40, 250 mM NaCl, 5 mM EDTA and 50 mM NaF) and the protein concentration was evaluated using a Rapid Gold BCA Protein Assay kit from Pierce (cat. no. A53225; Thermo Fisher Scientific, Inc.). Total proteins (50 μg) were loaded, separated via 10% SDS-PAGE and electrically transferred to PVDF membranes (cat. no. IPFL00010; EMD Millipore). Non-specific binding was blocked with 5% non-fat milk at room temperature for 1 h. Then, the proteins were incubated with the indicated antibodies at 4˚C overnight, followed by incubation with secondary antibodies (IRDye® 800CW conjugated goat anti-mouse IgG; 1:1,000; cat. no. 925-32210; LI-COR Biosciences) at room temperature for 1 h in the dark. Proteins were scanned and quantified using an Odyssey Infrared Imaging System (Odyssey version 3.0 Software; LI-COR Biosciences) in a blinded manner, and target proteins were normalized to GAPDH or the corresponding total proteins.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from fibroblasts using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and transcribed to cDNA using a Maxima First Strand cDNA Synthesis kit (Roche) according to the manufacturer’s protocols. Levels of mir-216a were detected using a BulgeLoop® miRNA RT-qPCR System (Guangzhou RiboBio Co., Ltd.). The thermocycling conditions were as follows: 95˚C for 10, then 40 cycles of 95˚C for 2 sec, 60˚C for 20 sec and 70˚C for 10 sec. The data were analyzed using the 2−ΔΔCT method as previously described (29). Total mRNA levels were normalized to GAPDH, and mir-216a
levels were normalized to U6. The primer sequences were as follows: Mouse collagen 1 (Col 1), forward, 5'-AGG CTT CAG TG G T T T G G A T G -3' and reverse, 5'-CACCCAACAGCACCATCGTTA-3'; mouse collagen 3 (Col 3), forward, 5'-CCC AACCAGATCCCAT-3' and reverse, 5'-GAACGACAG GAGCGTTGTA-3'; mouse connective tissue growth factor (Ctgf), forward, 5'-TGTTGATGAGCCAAGGAC-3' and reverse, 5'-AGTTGGCTCAGTCATAGTTG-3'; mouse fibronectin (Fn), forward, 5'-CCGGTGGCTGCTGAAGGACGCA-3' and reverse, 5'-CCGTTCCACGTGCTATTTATC-3'; mouse miR-216a, forward, 5'-CATGATCAGCTGGCGCCAGACACA GTTGCCAGCTG-3' and reverse, 5'-TAATCTCAGCTGGCA A-3'; mouse GAPDH, forward, 5'-CTGCCGCGCTGAG AACC-3' and reverse, 5'-TGGAGAGTGGGATTGTCTGT TG-3'; and U6, forward, 5'-CTGCTTCCAGCACAGCA-3' and reverse, 5'-AAGCCTTCAGGAATTTCCGTG-3'.

Immunofluorescence staining. Immunofluorescence staining was performed to detect the expression of the myofibroblast transdifferentiation biomarker α-smooth muscle actin (α-SMA) in mouse cardiac fibroblasts, as previously described (30). In brief, cardiac coverslips were fixed with 4% paraformaldehyde for 15 min at room temperature and then permeabilized with 0.2% Triton X-100, followed by incubation with 10% goat serum (GeneTex, Inc.) for 1 h at room temperature. The cells were then incubated with anti-α-SMA (1:100; cat. no. ab5694; Abcam) at 4˚C overnight, followed by incubation with the secondary antibody for 1 h at room temperature. The nuclei were stained with DAPI at room temperature for 30 sec. Images were captured using an Olympus DX51 fluorescence microscope (magnification, x400; Olympus Corporation) and quantified using Image-Pro Plus 6.0 (Media Cybernetics, Inc.). A total of 10-15 fields of view were observed per coverslip.

Bioinformatic prediction. The online database TargetScanMouse (Release 7.1, http://www.targetscan.org/mmu_71/) was employed for target prediction and analysis of miR-216a (31).

Statistical analysis. Results were presented as the mean ± standard error of the mean. Unpaired Student’s t-tests (two-tailed) were used to compare differences between two groups. One-way ANOVA followed by a Tukey’s post hoc test was performed to determine differences across multiple groups. All data were analyzed using SPSS 22.0 software (IBM Corporation) and considered to indicate a statistically significant difference.

Results

miR-216a inhibition attenuates TGF-β-induced myofibroblast transdifferentiation. Alterations in the expression of miR-216a were evaluated in TGF-β-treated adult mouse cardiac fibroblasts. As presented in Fig. 1A, TGF-β incubation increased miR-216a levels in a dose-dependent manner. In addition, miR-216a levels were increased in a time-dependent manner, albeit with no significant difference between 24 and 48 h (Fig. 1B). Therefore, a 24-h treatment period with 10 ng/ml of TGF-β was selected for further experiments. An antagonist was used to inhibit miR-216a expression in cultured adult mouse cardiac fibroblasts; the efficiency of transfection was demonstrated via RT-qPCR analysis (Fig. 1C). As presented in Fig. 1D, miR-216
miR-216a activation exacerbes TGF-β-induced myofibroblast transdifferentiation. Next, the present study investigated whether miR-216a overexpression promoted myofibroblast transdifferentiation in response to TGF-β. Cardiac fibroblasts were transfected with a miR-216a agomir (Fig. 2C), and it was observed that miR-216a upregulation enhanced the TGF-β-induced increase in the expression of fibrotic markers compared with the control (Fig. 2A). In addition, it was determined that the miR-216a agomir further promoted α-SMA expression in response to TGF-β compared with the control (Fig. 2B). Collectively, these findings suggested that miR-216a is required for the induction of myofibroblast transdifferentiation.

The present study, however, the results demonstrated that miR-216a inhibition did not significantly affect the phosphorylation of Smad3 (Fig. 3A and B). TGFBR2 is the primary receptor of TGF-β and mediates its profibrotic effect. Furthermore, bioinformatics analysis using TargetScan indicated that the TGFBR2 gene may be a target for miR-216a (data not shown). Therefore, the expression levels of TGFBR2 were analyzed via western blotting; however, the protein levels of TGFBR2 were not altered by the up- or downregulation of miR-216a, with or without TGF-β treatment (Fig. 3A, C, E and F).

In addition to the canonical Smad-dependent signaling, TGF-β also activates Smad-independent pathways, including the AKT pathway (8). Previous studies have reported that AKT and its downstream target GSK3β also contribute to the regulation of myofibroblast transdifferentiation (8,32). Therefore, the phosphorylation status of AKT and GSK3β was examined in the present study, and the results demonstrated that the miR-216a agomir significantly inhibited TGF-β-induced AKT/GSK3β activation compared with the control (Fig. 3D); by contrast, the miR-216a agomir further promoted AKT/GSK3β phosphorylation following treatment with TGF-β (Fig. 3G). Additionally, inhibition of AKT with MK2206 significantly attenuated the miR-216a agomir-mediated increase in myofibroblast transdifferentiation in response to TGF-β stimulation, as determined by the decreased mRNA levels of Col 1 and Col 3 (Fig. 3H). Collectively, these results indicated that miR-216a promoted TGF-β-induced myofibroblast transdifferentiation via activating AKT/GSK3β.

miR-216a activates AKT via inhibition of PTEN. Finally, the possible mechanisms underlying the miR-216a-mediated
miRNAs exert biological regulation on various pathophysiological procedures via complementary binding to cognate mRNA transcripts, and subsequent degradation of the targeted transcripts (12). Among the miR‑216a target genes predicted using TargetScan was Pten, a negative regulator of AKT signaling (Fig. 4A) (33). It was observed that the miR‑216a agomir further decreased PTEN expression in response to TGF‑β in cardiac fibroblasts (Fig. 4B), while miR‑216a antagomir significantly attenuated TGF‑β‑induced PTEN reduction compared with the control (Fig. 4C). To determine the role of PTEN in AKT activation by miR‑216a, the expression of PTEN was knocked down in cardiac fibroblasts using siPten (Fig. 4D). RT‑qPCR analysis revealed that PTEN knockdown abolished the miR‑216a agomir‑mediated acceleration of myofibroblast transdifferentiation, and that miR‑216a activated AKT via the inhibition of PTEN. The results indicated that miR‑216a is involved in myofibroblast transdifferentiation, and that targeting of miR‑216a may aid the development of efficacious interventions to treat cardiac fibrosis.

In response to mechanical or neurohumoral stimulation, cardiac fibroblasts transdifferentiate into myofibroblasts that produce large amounts of ECM and trigger the fibrotic process (1). Additionally, myofibroblasts secrete various factors that accelerate cardiac remodeling via autocrine and paracrine pathways (34). Nagpal et al (17) demonstrated that inhibiting the fibroblast-to-myofibroblast transition is required for the treatment of human cardiac fibrosis. miRNAs are now considered to be important regulators of gene expression in various pathophysiological processes (11). Previous studies have reported that miRNAs are specifically involved in the regulation of cardiac fibrosis (15‑17). The present data demonstrated that an miR‑216a agomir enhanced TGF‑β‑induced myofibroblast transdifferentiation, whereas an miR‑216a antagomir inhibited this process and decreased ECM synthesis. It was previously reported that miR‑216a was upregulated in TGF‑β‑treated mouse glomerular activity of AKT were investigated. miRNAs exert biological regulation on various pathophysiological procedures via complementary binding to cognate mRNA transcripts, and subsequent degradation of the targeted transcripts (12). Among the miR‑216a target genes predicted using TargetScan was Pten, a negative regulator of AKT signaling (Fig. 4A) (33). It was observed that the miR‑216a agomir further decreased PTEN expression in response to TGF‑β in cardiac fibroblasts (Fig. 4B), while miR‑216a antagomir significantly attenuated TGF‑β‑induced PTEN reduction compared with the control (Fig. 4C). To determine the role of PTEN in AKT activation by miR‑216a, the expression of PTEN was knocked down in cardiac fibroblasts using siPten (Fig. 4D). RT‑qPCR analysis revealed that PTEN knockdown abolished the miR‑216a agomir‑mediated acceleration of myofibroblast transdifferentiation, as determined by the mRNA levels of Col 1 and Col 3 (Fig. 4E). Thus, the present data suggested that miR‑216a may activate AKT by inhibiting PTEN.

**Discussion**

Myofibroblast transdifferentiation enhances collagen synthesis and is responsible for the occurrence of cardiac fibrosis; however, there is no available strategy to effectively suppress this pathological process (1,27). In the present study, it was observed that miR‑216a expression was upregulated in TGF‑β‑treated cardiac fibroblasts, which in turn activated the AKT/GSK3β signaling pathway and induced myofibroblast transdifferentiation. Furthermore, it was revealed that AKT inhibition abolished the miR‑216a agomir‑mediated acceleration of myofibroblast transdifferentiation, and that miR‑216a activated AKT via the inhibition of PTEN. The results indicated that miR‑216a is involved in myofibroblast transdifferentiation, and that targeting of miR‑216a may aid the development of efficacious interventions to treat cardiac fibrosis.
mesangial cells, leading to glomerular mesangial cell survival and hypertrophy (18). Additionally, it was observed that miR-216a mediated TGF-β-induced collagen expression in kidney cells (23). The heart comprises numerous types of cells; previous studies have identified important roles for various other cell types in the regulation of cardiac fibrosis, in addition to cardiac fibroblasts. For example, activation of M2 macrophages is associated with cardiac fibrosis (35,36). Yang et al. (22) demonstrated that miR-216a promoted M1 macrophage polarization via Smad3 activation. The present study demonstrated that transfection with an miR-216a antagonist did not affect Smad3 phosphorylation, but it increased the phosphorylation of AKT/GSK3β. These results suggested that the functional effects of miR-216a are cell type-dependent, and that upregulation of miR-216a in cardiac fibroblasts may lead to cardiac fibrosis. These data collectively provided rationale for the treatment of myofibroblast transdifferentiation and cardiac fibrosis via the targeting of miR-216a in cardiac fibroblasts.

AKT/GSK3β signaling serves an important role in the pathological fibrotic response (8). AKT is phosphorylated and activated in response to fibrotic stimulation, and AKT inhibition alleviates pressure overload-induced cardiac fibrosis (37). Activated AKT phosphorylates and inactivates GSK3β, which is also an important regulator of cardiac fibrosis (32). Lal et al. (32) previously demonstrated that GSK3β physically interacted with Smad3, inhibiting its transcriptional activity. Specific deletion of GSK3β in cardiac fibroblasts induced a profibrotic myofibroblast phenotype and contributed to the pathogenesis of cardiac fibrosis post-myocardial infarction (32). In the present study, it was revealed that an miR-216a agomir further enhanced TGF-β-induced AKT/GSK3β phosphorylation; conversely, an miR-216a antagonist inhibited AKT/GSK3β activation. Inhibition of AKT attenuated the miR-216a agomir-mediated promotion of myofibroblast transdifferentiation. Of note, an alteration in Smad3 phosphorylation was not observed, suggesting that the AKT/GSK3β pathway also contributes to fibrotic regulation, independent of Smad3. TGFBR2 has been identified as the primary receptor of TGF-β, and it delivers its profibrotic signal from the cell membrane into the cytoplasm (38); however, it was revealed that TGFBR2 protein levels were unchanged following transfection with the agomir or antagonist of miR-216a, with or without TGF-β. Collectively, these data indicated that the effects of miR-216a on myofibroblast transdifferentiation may be specifically mediated by the downstream, non-canonical AKT/GSK3β signaling pathway independent of TGFBR2. PTEN is the main negative regulator of AKT signaling, and its inhibition results in the accumulation of phosphatidylinositol(3,4,5)-trisphosphate, mimicking the effect of PI3K activation and inducing the activation of downstream AKT signaling (33). In the present study, it was
observed that miR-216a was predicted to bind the 3'-UTR of Pten, thus potentially leading to the downregulation of PTEN protein levels and subsequent activation of AKT. Functional experiments confirmed that transfection with the miR-216a agonist decreased PTEN levels in combination with TGF-β, whereas the miR-216a antagonist alleviated the TGF-β-induced downregulation of PTEN. Pten knockdown attenuated the beneficial effects of the miR-216a antagonist. Nie et al (39) recently reported that PTEN downregulation by miR-217 enhances the proliferation of fibroblasts and accelerates collagen synthesis. In the present study, it was demonstrated that downregulation of PTEN by miR-216a promoted fibrotic progress via activation of the downstream AKT/GSK3β pathway.

In conclusion, it was revealed that miR-216a exacerbated TGF-β-induced myofibroblast transdifferentiation via the PTEN-dependent activation of AKT/GSK3β signaling. The present study identified roles and underlying mechanisms of miR-216a in myofibroblast transdifferentiation, suggesting that miR-216a may be a novel therapeutic target for the treatment of cardiac fibrosis.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Nature Science Foundation of Hubei Province (grant no. 2014CFA061), the Foundation Research Funds for the Central Research Funds for the Central Universities, China (grant nos. 2042016k0082 and 2042017k0158) and the Major Program of Technological Innovation of Hubei Province (grant no. 2016ACA153).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

CQ and BY contributed to the conception and design of the experiments. XL, TY, LW, SL and XZ performed the experiments. CQ, GW, JL and SS analyzed the experimental results and interpreted the data. CQ and BY drafted and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All of the animal experimental protocols were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (approval no. 20171003).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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