Int J Clin Exp Pathol 2016;9(3):2795-2802
www.ijcep.com /ISSN:1936-2625/IJCEP0018859

Original Article

Expression of miR-21 is involved in myocardial infarction through the IL-6/STAT3 and Bcl-2/caspase-3 pathway

Hao Sun, Jun Cai, Li Xu, Jiamei Liu, Mulei Chen, Meili Zheng, Lefeng Wang, Xinchun Yang

Heart Center, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China

Received October 30, 2015; Accepted December 26, 2015; Epub March 1, 2016; Published March 15, 2016

Abstract: To investigate the roles of miR-21 expression is involved in acute myocardial infarction (AMI) through IL-6/STAT3 and Bcl-2/caspase-3 pathway. The expression of miR-21 in patients with AMI is higher than that of normal volunteer. The CK-MB, and cTnI concentrations of higher miR-21 group in patients with AMI was lower than those of lower miR-21 group. H9c2 cells were co-cultured with H_2O_2 (100 μmol/L) was deed as AMI model in vitro. Over-expression of miR-21 could decrease malondialdehyde (MDA) and NO concentrations and IL-6 level, and increased glutathione peroxidase (GSH-PX), glutathione (GSH), superoxide dismutase (SOD) concentrations in H9c2 cells exposed by H_2O_2. Meanwhile, over-expression of miR-21 could suppressSTAT3 protein expression, activated Bcl-2 protein expression, and decreased caspase-3 activity in H9c2 cells exposed by H_2O_2. In conclusion, the expression of miR-21 is involved in AMI through the IL-6/STAT3 and Bcl-2/caspase-3 pathway.

Keywords: miR-21, acute myocardial infarction, IL-6, STAT3, Bcl-2, caspase-3

Introduction

Heart failure is the end stage of various cardiovascular diseases with high mortality rate [1]. According to the epidemiological survey, the incidence of heart failure is nearly 2% among the world and the prevalence rate of heart failure is 0.9% among 35 to 74 year-old adults in China, namely, the patients with heart failure reach 3.6 million, and the annual mortality rate is between 20% and 50% reaching reached 67% for five years [2, 3]. Clinical studies have confirmed that cardiac remodeling is the important pathological basis for the attack and development of heart failure and the key step in the evolution of cardiac function form compensation to decompensation [4].

Widely existed in cells, miRNA is the small RNA regulating the genetic expression horizontally after transcription. It maintains the normal function of the cells in many aspects, such as maintaining self-renewal and directional differentiation of embryonic stem cell, enhancing the mitochondrial translation during muscle differentiation process, regulating T cells and macrophages responses; while on the other hand, the abnormal expression of miRNA is closely related to many diseases, such as cardiovascular disease and abnormal biological clock [5-7].

Cardiovascular disease is one of the serious diseases that threaten human health, particularly; the acute myocardial Infarction (AMI) has become an important cause for the increasing death rate of cardiovascular diseases [8]. Therefore, early, timely and accurate diagnosis of AMI and estimation of disease state are essential for the proactive and effective treatment and the morality deduction of cardiac reperfusion in time [9]. The early treatment of AMI plays a vital role in the prognosis of patients. At present, the serum biomarkers in clinical diagnosis of AMI iscreatine kinse isoenzyme, myoglobin, and muscle protein etc. which have reached a certain level of sensitivity and specificity in the early diagnosis of AMI, but the researchers continue the research of new serum markers with high sensitivity and specificity, especially for those can well reflect the prognosis of patients with AMI [10]. The new discovery of non-coding microRNA (miRNA) in the past ten years is closely related to the occurrence, development and prognosis of vari-

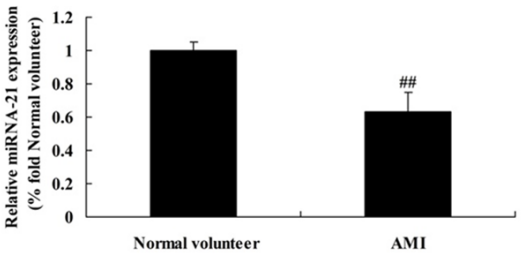

Figure 1. The expression of miR-21 is involved in Patients with AMI. Normal volunteer, Normal volunteer group; AMI, Patients with AMI group. ##P<0.01 versus Normal volunteer group.

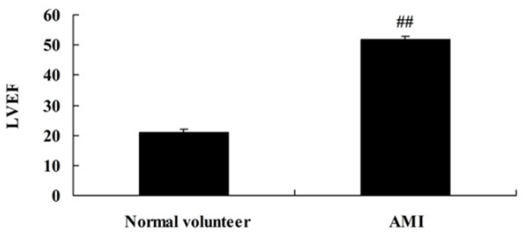

Figure 2. The level of LVEF is involved in the expression of miR-21 ofpatients with AMI. ##P<0.01 versus Normal volunteer group.

ous diseases [11]. The expression of miRNA has good tissue specificity and high stability in blood, thus, it can be speculated that miRNA of myocardial specificity may be an ideal biomarker for early diagnosis of AMI [12]. Besides, miRNA also plays an important role in the pathophysiologic development of myocardial infarction, myocardial fibrosis after infarction and cardiac remodeling, etc [13]. In the present study we investigated whether the expression of miR-21 is involved in myocardial infarction through the IL-6/STAT3 pathway and Bcl-2/caspase-3.

Materials and methods

Patients' characteristics and serum histamine determination

Serum was taken from patients with AMI who were admitted to Beijing Chao-Yang Hospital, Capital Medical University. Thereinto, 52 patients with AMI and 20 patients without AMI (normal volunteer) were selected in this study. Patients received and detected LVEF, creatine kinase (CK)-MB, and cTnI.

Myocardial infarction cell model

Rat Cardiac Myocytes H9c2 cell was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) and was maintained in 5% CO_2 at 37°C in RPMI-1640 medium (Gibco Life Technologies, Los Angeles, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco Life Technologies, Los Angeles, CA, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco Life Technologies, Los Angeles, CA, USA). H9c2 cell was co-cultured with H_2O_2 (100 μmol/L) was deed as AMI model in vitro.

Transfection of miRNA-21

H9c2 cells co-cultured with H_2O_2 were seeded into 6-well plates and transfected with anti-miR negative control or miRNA-21 using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h of transfection, the next step experiment was executed.

Measurement ofCK, CK-MB, malondialdehyde (MDA), glutathione peroxidase (GSH-PX), glutathione (GSH), superoxide dismutase (SOD), nitric oxide (NO) and interleukine(IL)-6

Blood was obtained and used to detect the levels of CK and CK-MB using Commercial kits (Jiancheng Technology Co., Ltd., Nanjing, China). After 24 h of transfection, H9c2 cells was obtained and used to detect the levels of MDA, GSH-PX, GSH, SOD, NO and IL-6 using Commercial kits (Jiancheng Technology Co., Ltd., Nanjing, China).

Quantitative reverse transcription-PCR (qRT-PCR)

After 24 h of transfection, Total RNA was obtained from blood or H9c2 cells and isolated by Trizol (Invitrogen) according to the manufacturer's recommendations. 2 μg total RNA was used to reverse transcriptase cDNA using MMLV RT (Promega Corp. Madison, WI, USA). MiR-21-Forward: 5'-ACACTCCAGCTGGGTAGCTTATCAGACTGA-3' and 5'-TGGTGTCGTGGAGTCG-3'; U6-Forward: 5'-CGCTTCGGCAGCACATATACTAAAATTGGAAC-3' and 5'-GCTTCACGAATTTGCGTGTCATCCTTGC-3'.

Western blots

After 24 h of transfection, H9c2 cells was obtained and used to extract protein using an ice-cold lysed in RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific Inc., Rockford, USA). Protein concentrations were measured

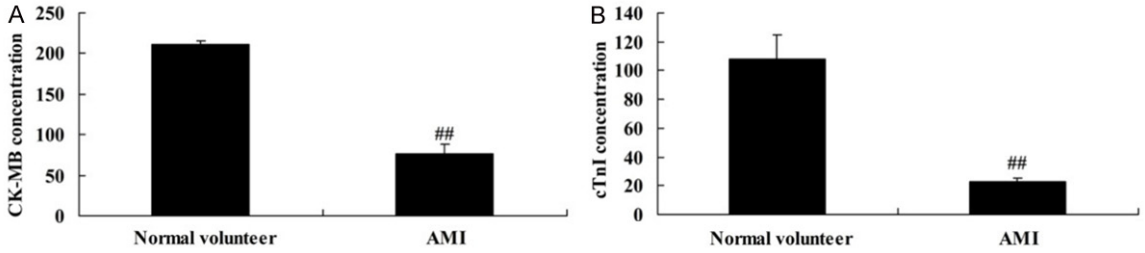

Figure 3. CK-MB and cTnI concentrations are involved in the expression of miR-21 of patients with AMI. ##P<0.01 versus Normal volunteer group.

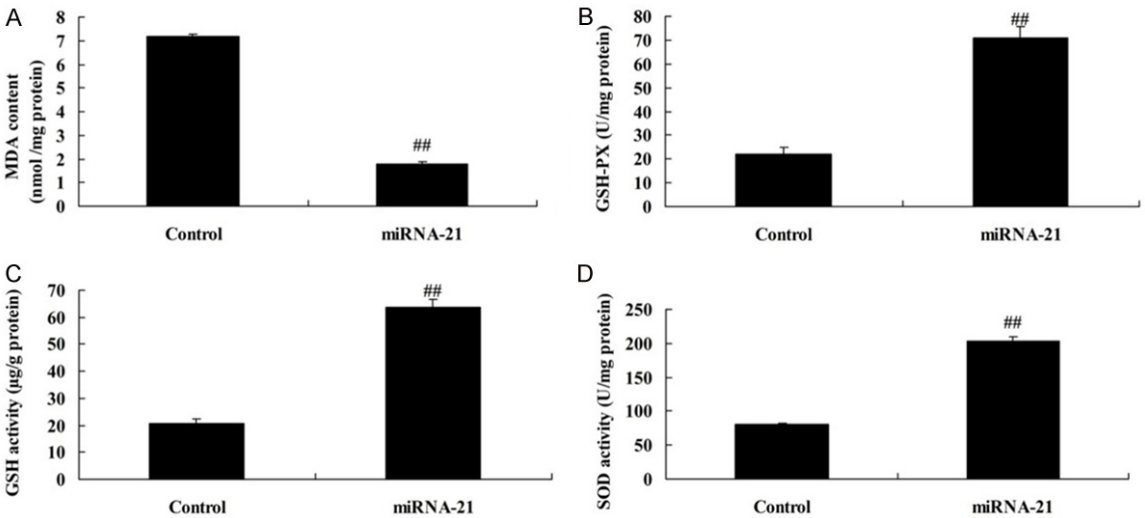

Figure 4. The MDA, GSH-PX, GSH, SOD concentrations is involved in miR-21. The MDA (A), GSH-PX (B), GSH (C), SOD (D) concentrations is involved in rat with AMI. Control, control group; miRNA-21, over-expression of miRNA-21 group. ##P<0.01 versus control group.

with the BCA Protein Assay (Beyotime Institute of Biotechnology, Shanghai, China). 80 μg proteins were separated by 10% sodium dodecyl SDS-polyacrylamide gels (Beyotime Institute of Biotechnology) and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). Membranes were detected using phosphorylation of STAT3 (p-STAT3,1:3000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), Bcl-2 (1:3000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), caspase-3 (1:3000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and β-actin (1:3000, Beyotime Institute of Biotechnology, Shanghai, China) at 4°C overnight. After additional incubation with horseradish peroxidase-conjugated goat antimouse antibody for 1 at room temperature, the immune complexes were detected by enhanced chemiluminescence (Cell Signaling Technology). The optical densities of immunopositive bands were determined Gene Tools analysis software.

Statistical analyses

Data are presented as the mean ± standard deviation and analyzed with the Statistical Package SPSS, version 12.0 for Windows (SPSS, Inc., Chicago, IL, USA). To analyze the data statistically, we performed one-way ANOVA with post hoc analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of miR-21 is involved in patients with AMI

The results from the qRT-PCR assay, demonstrated that miR-21 expression was significant-

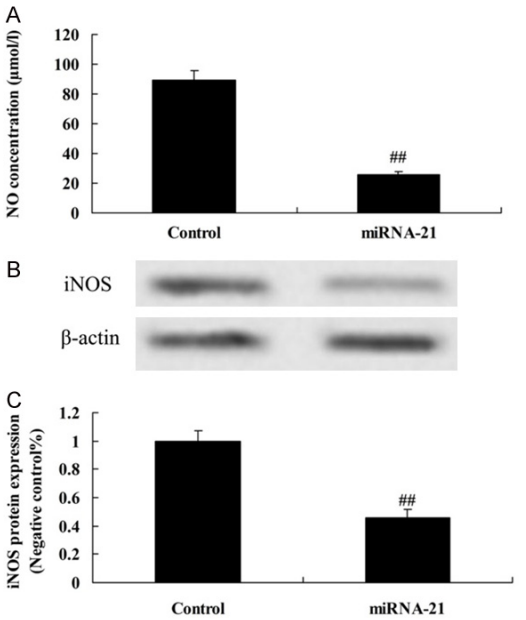

Figure 5. NO concentrations and iNOS protein expression are involved in miR-21. NO concentration (A) and iNOS protein expression (B) using Western blot analysis, statistical analysis of iNOS protein expression (C) are involved in rat with AMI. Control, control group; miRNA-21, over-expression of miRNA-21 group. ##P<0.01 versus control group.

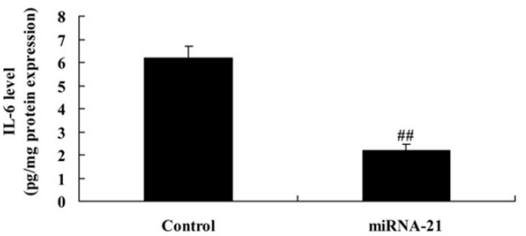

Figure 6. The IL-6 level is involved in miR-21. Control, control group; miRNA-21, over-expression of miRNA-21 group. ##P<0.01 versus control group.

ly lower in patients tissues with AMI than that ofnormal volunteer, suggesting that miR-21 expression is involved in AMI (**Figure 1**).

Level of LVEF is involved in the expression of miR-21 of patients with AMI

In order to investigate the association between the level of LVEF and the expression of miR-21 in patients with AMI, LVEF level and miR-21 expression in these samples was evaluated.As illustrated in **Figure 2**, the level of LVEF in miR-21 lowerof patients with AMI was lower than that of miR-21 higher of patients with AMI.

CK-MB and cTnI concentrations are involved in the expression of miR-21 of patients with AMI

In order to determine the association betweenCK-MB and cTnI concentrations and the expression of miR-21 in patients with AMI, CK-MB and cTnI concentrations in these samples was evaluated. These CK-MB and cTnI concentrations of miR-21 lower of patients with AMI were higher than those of miR-21 higher of patients with AMI (**Figure 3**).

MDA, GSH-PX, GSH and SOD concentrations is involved in miR-21

To investigate the association between oxidative stress and the expression of miR-21 in H9c2 cells co-cultured with H_2O_2, the MDA, GSH-PX, GSH and SOD concentrations were inspected. In over-expression of miR-21 group, MDA concentration was significantly reduced, and GSH-PX, GSH and SOD concentrations were significantly increased, as compared with control group (**Figure 4**).

NO concentrations and iNOS protein expression are involved in miR-21

To further determine the association between NO and miR-21 expression in H9c2 cells co-cultured with H_2O_2, NO concentrations and iNOS protein expression were detected. As illustrated in **Figure 5**, over-expression of miR-21 significantly reduced NO concentrations and the protein expression of iNOS, as compared with control group.

IL-6 level is involved in miR-21

To confirm the association between IL-6 level and miR-21 expression in H9c2 cells, IL-6 activity level was measured after H9c2 cells transfected. As showed in **Figure 6**, over-expression of miR-21 could significantly reduced IL-6 activity level in H9c2 cells, as compared with control group.

STAT3 level is involved in miR-21

To further confirm the association between STAT3 level and miR-21 expression in H9c2 cells transfected, p-STAT3 protein expression was measured using Western blots. Transfection of H9c2 cells showed p-STAT3 protein expression was significantly inhibited in over-expression of miR-21, as compared with control group (**Figure 7**).

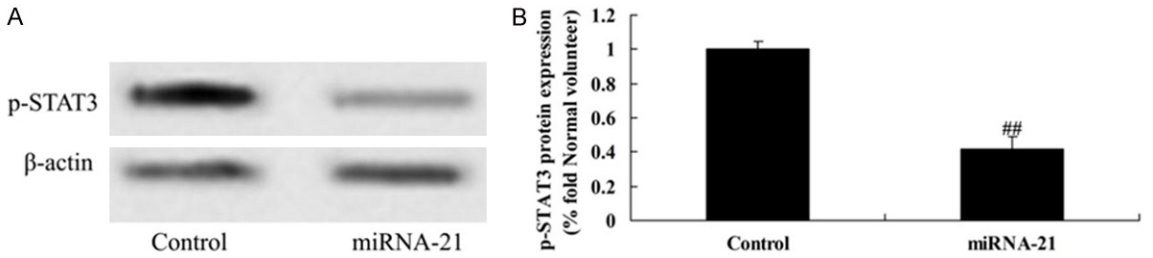

Figure 7. The STAT3 level is involved in miR-21. iNOS protein expression (A) using Western blot analysis, statistical analysis of iNOS protein expression (B) isinvolved in rat with AMI. Control, control group; miRNA-21, over-expression of miRNA-21 group. ##P<0.01 versus control group.

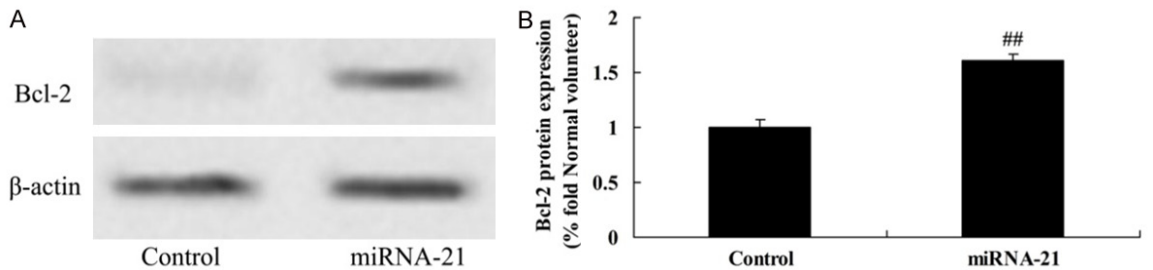

Figure 8. The Bcl-2 level is involved in miR-21. Bcl-2 protein expression (A) using Western blot analysis, statistical analysis of Bcl-2 protein expression (B) isinvolved in rat with AMI. Control, control group; miRNA-21, over-expression of miRNA-21 group. ##P<0.01 versus control group.

Bcl-2 level is involved in miR-21

To investigate the apoptosis regulation of miR-21 on AMI, Bcl-2 protein expression was detected using Western blots after H9c2 cells transfection. Combined with over-expression of miR-21, Bcl-2 protein expression was significantly promoted in control group (**Figure 8**).

Caspase-3 level is involved in miR-21

To further investigate the effects of caspase-3 regulation of miR-21 on AMI, H9c2 cells transfectionwas used to analyze the protein expression of p-STAT3. Relative to control H9c2 cells,caspase-3 activity was effectively suppressed in over-expression of miR-21 (**Figure 9**).

Discussion

Myocardial infarction (MI), characterized by acute attack, multi complication and high fatality rate, is a common critical and severe disease in coronary artery disease [14]. In 1970's, some scholars proposed the concept of ventricular remodeling after myocardial infarction; it has become a hot topicin the research of ventricular system [15]. Formation of left ventricular and serious injury to the ventricular function of the patients with myocardial infarction and significantly increase complication and mortality rate [5].

Myocardial cell apoptosis, ventricular remodeling and myocardial fibrosis are the important pathophysiological processes of lesion repair and entire compensation of myocardial cell after myocardial infarction [16]. MiRNA adjust the adaption and the non-benign gene expression ways mainly through the cell growth or apoptosis, extracellular matrix remodeling and the activation of neuroendocrine [17].

Hypoxia can trigger a series of responses from myocardial cell to relieve the cell hypoxia and cause irreversible damage. These responses include the proliferation, migration and angiogenesis of endothelial cell and activity termination and apoptosis of cells as well [18]. During hypoxia, multiple cellular pathways are activated to resume the balance and stability of oxygen supply. Hypoxia-inducible factor plays a key role in stimulating new blood vessels by regulating endothelial growth factor and angiogen-

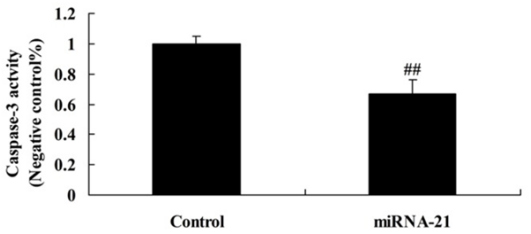

Figure 9. The caspase-3 level is involved in miR-21. Control, control group; miRNA-21, over-expression of miRNA-21 group. ##P<0.01 versus control group.

esis promoting protein factor-2 [19]. It is generally considered that miR-21 is the most important miRNA in the regulation of hypoxia [20]. The research has found that the expression of miR-21 increases and the expression in all cells and tissues are increased; therefore, miR-21 is regarded as the main regulatory genes [21]. In the present study, the association between miR-21 expression and LVEF level, CK-MB and cTnI concentrations in patients with AMI.

A large number of inflammatory cytokines, including TNF-a and IL-6, are produced in a short time in the early stage of AMI through mechanical deformation of myocardial cell, ischemia stimulation, oxygen free radical damage, self-amplification of cell factor and other mechanisms [22]. The generation and increasing of inflammatory cytokines is a kind of endogenous stress reaction to myocardial injury [23]. Inflammatory reaction is a necessary for wound healing and scar formation. The activated inflammatory cytokine induce cardiac muscle tissue to have corresponding pathological changes, including the hypertrophy and apoptosis of cardiomyocyte, extracellular matrix changes and contraction of disabilities [24]. TNF-α plays an important role in these process and the experiments have confirmed that IL-6 has inhibitory effect on myocardial contractility and the effect increase with the dosage, besides, IL-6 also can cause myocardial hypertrophy and the effect of anti-apoptosis on myocardial cells [25, 26]. We found that over-expression of miR-21 significantly reduced oxidative stress, NO concentrations and iNOS protein expression and IL-6 activity level in H9c2 cells. Hutcheson et al. concluded that miR-21-mediated decreased neutrophil apoptosis is a determinant of impaired coronary collateral growth in metabolic syndromethrough suppression of oxidative stress [27]. Laatikainen et al. reported that SOD3 decreases ischemic injury derived apoptosis through miR-21 and iNOS [28]. Zhou et al. showed that miR-21 suppressed neuronal apoptosis through suppressing IL-6 after peripheral nerve injury [29].

Previous studies have found that the STAT3 pathway is thekeyto the ischemic preconditioning of myocardial preservation, particularly for the pretreatment of second window of protection mechanism, ischemic preconditioning can activate the STAT3 pathway immediately, upregulate the inhibitor of apoptosis protein by gene expression, reduce the area of myocardial infarction and inhibit the myocardial cell apoptosis by myocardial protective substance [30, 31]. It is found that myocardial protective effect of ischemic post conditioning is invalid for the rats with STAT3 gene knocked out [32]. In this study, we found that over-expression of miR-21 significantly inhibited p-STAT3 protein expression, promoted Bcl-2 protein expression and suppressed caspase-3 activity in H9c2 cells transfected. Haider et al. revealed that miR-21 is a key determinant in preconditioning of skeletal myoblasts through IL-11/Stat3 anti-apoptotic pathway [33]. Han et al. reported that miR-21 suppressed apoptosis of cortical neurons in traumatic brain injury through Bcl-2, caspase-3 and caspase-9 [27].

In conclusion, the expression of miR-21 is involved in LVEF level, CK-MB and cTnI concentrations in patients with AMI, and miR-21 may plays a protective role against AMI through the IL-6/STAT3 and Bcl-2/caspase-3 pathway. Thus, IL-6/STAT3 and Bcl-2/caspase-3 pathway regulated by miR-21 should be used cautiously in AMI, until further clinical and basic studies are completed.

Disclosure of conflict of interest

None.

Address correspondence to: Xinchun Yang, Heart Center, Beijing Chao-Yang Hospital, Capital Medical University, 8 Gong-Ti South Road, Beijing, China. Tel: 86-10-85231066; Fax: 86-10-65951064; E-mail: XinchunYang126@163.com

References

[1] Yang J, Ma H, Liu J, Wang C, Shi Y, Xie H, Huo F, Liu F and Lin K. Delayed-enhancement magnetic resonance imaging at 3.0T using 0.15 mmol/kg of contrast agent for the assessment

of chronic myocardial infarction. Eur J Radiol 2014; 83: 778-782.
[2] Lotz C, Ritter O and Muellenbach RM. Assisted beating of the ischemic heart: how to manage the pulseless ST–segment-elevation myocardial infarction patient. Circulation 2014; 130: 1095-1104.
[3] Geng W, Tian X, Fu X, Wang P, Wang Y, Wang X, Li W and Liu X. Early routine angioplasty versus selective angioplasty after successful thrombolysis in acute ST-segment elevation myocardial infarction. Coron Artery Dis 2013; 24: 238-243.
[4] Han Y, Guo J, Zheng Y, Zang H, Su X, Wang Y, Chen S, Jiang T, Yang P, Chen J, Jiang D, Jing Q, Liang Z, Liu H, Zhao X, Li J, Li Y, Xu B and Stone GW. Bivalirudin vs heparin with or without tirofiban during primary percutaneous coronary intervention in acute myocardial infarction: the BRIGHT randomized clinical trial. JAMA 2015; 313: 1336-1346.
[5] Devaux Y, Vausort M, Goretti E, Nazarov PV, Azuaje F, Gilson G, Corsten MF, Schroen B, Lair ML, Heymans S and Wagner DR. Use of circulating microRNAs to diagnose acute myocardial infarction. Clin Chem 2012; 58: 559-567.
[6] Peng L, Chun-guang Q, Bei-fang L, Xue-zhi D, Zi-hao W, Yun-fu L, Yan-ping D, Yang-gui L, Wei-guo L, Tian-yong H and Zhen-wen H. Clinical impact of circulating miR-133, miR-1291 and miR-663b in plasma of patients with acute myocardial infarction. Diagn Pathol 2014; 9: 89.
[7] Liang J, Bai S, Su L, Li C, Wu J, Xia Z and Xu D. A subset of circulating microRNAs is expressed differently in patients with myocardial infarction. Mol Med Rep 2015; 12: 243-247.
[8] Nabialek E, Wanha W, Kula D, Jadczyk T, Krajewska M, Kowalowka A, Dworowy S, Hrycek E, Wludarczyk W, Parma Z, Michalewska-Wludarczyk A, Pawlowski T, Ochala B, Jarzab B, Tendera M and Wojakowski W. Circulating microRNAs (miR-423-5p, miR-208a and miR-1) in acute myocardial infarction and stable coronary heart disease. Minerva Cardioangiol 2013; 61: 627-637.
[9] Boon RA and Dimmeler S. MicroRNAs in myocardial infarction. Nat Rev Cardiol 2015; 12: 135-142.
[10] Hsu A, Chen SJ, Chang YS, Chen HC and Chu PH. Systemic approach to identify serum microRNAs as potential biomarkers for acute myocardial infarction. Biomed Res Int 2014; 2014: 418628.
[11] Matsumoto S, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, Kitamura T, Hamasaki T, Nanto S, Kawahara Y and Komuro I. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. Circ Res 2013; 113: 322-326.
[12] Huang S, Chen M, Li L, He M, Hu D, Zhang X, Li J, Tanguay RM, Feng J, Cheng L, Zeng H, Dai X, Deng Q, Hu FB and Wu T. Circulating MicroRNAs and the occurrence of acute myocardial infarction in Chinese populations. Circ Cardiovasc Genet 2014; 7: 189-198.
[13] Lippi G, Mattiuzzi C and Cervellin G. Circulating microRNAs (miRs) for diagnosing acute myocardial infarction: meta-analysis of available studies. Int J Cardiol 2013; 167: 277-278.
[14] He H, Li N, Zhao Z, Han F, Wang X and Zeng Y. Ischemic postconditioning improves the expression of cellular membrane connexin 43 and attenuates the reperfusion injury in rat acute myocardial infarction. Biomed Rep 2015; 3: 668-674.
[15] Huang Y, Qi Y, Du JQ and Zhang DF. MicroRNA-34a regulates cardiac fibrosis after myocardial infarction by targeting Smad4. Expert Opin Ther Targets 2014; 18: 1355-1365.
[16] Chen X, Zhang L, Su T, Li H, Huang Q, Wu D, Yang C and Han Z. Kinetics of plasma microRNA-499 expression in acute myocardial infarction. J Thorac Dis 2015; 7: 890-896.
[17] Kang HJ, Kang WS, Hong MH, Choe N, Kook H, Jeong HC, Kang J, Hur J, Jeong MH, Kim YS and Ahn Y. Involvement of miR-34c in high glucose-insulted mesenchymal stem cells leads to inefficient therapeutic effect on myocardial infarction. Cell Signal 2015; 27: 2241-2251.
[18] Li S, Fan Q, He S, Tang T, Liao Y and Xie J. MicroRNA-21 Negatively Regulates Treg Cells Through a TGF-beta1/Smad-Independent Pathway in Patients with Coronary Heart Disease. Cell Physiol Biochem 2015; 37: 866-878.
[19] Dong X, Liu S, Zhang L, Yu S, Huo L, Qile M, Liu L, Yang B and Yu J. Downregulation of miR-21 is involved in direct actions of ursolic acid on the heart: implications for cardiac fibrosis and hypertrophy. Cardiovasc Ther 2015; 33: 161-167.
[20] Cardin S, Guasch E, Luo X, Naud P, Le Quang K, Shi Y, Tardif JC, Comtois P and Nattel S. Role for MicroRNA-21 in atrial profibrillatory fibrotic remodeling associated with experimental postinfarction heart failure. Circ Arrhythm Electrophysiol 2012; 5: 1027-1035.
[21] Liu X, Dong Y, Chen S, Zhang G, Zhang M, Gong Y and Li X. Circulating MicroRNA-146a and MicroRNA-21 Predict Left Ventricular Remodeling after ST-Elevation Myocardial Infarction. Cardiology 2015; 132: 233-241.
[22] Zamilpa R, Ibarra J, de Castro Bras LE, Ramirez TA, Nguyen N, Halade GV, Zhang J, Dai Q, Dayah T, Chiao YA, Lowell W, Ahuja SS, D'Armiento J, Jin YF and Lindsey ML. Transgenic overexpression of matrix metalloproteinase-9 in macrophages attenuates the inflammatory response and improves left ventricular function post-myocardial infarction. J Mol Cell Cardiol 2012; 53: 599-608.

[23] Sato T, Kameyama T, Noto T, Nakadate T, Ueno H, Yamada K and Inoue H. The impact of anti-inflammatory cytokines provoked by CD163 positive macrophages on ventricular functional recovery after myocardial infarction. J Thromb Thrombolysis 2014; 37: 139-147.

[24] Saevarsdottir S, Oskarsson OO, Aspelund T, Eiriksdottir G, Vikingsdottir T, Gudnason V and Valdimarsson H. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. J Exp Med 2005; 201: 117-125.

[25] Asgeri M, Pourafkari L, Kundra A, Javadzadegan H, Negargar S and Nader ND. Dual effects of tumor necrosis factor alpha on myocardial injury following prolonged hypoperfusion of the heart. Immunol Invest 2015; 44: 23-35.

[26] Cho E, Kim M, Ko YS, Lee HY, Song M, Kim MG, Kim HK, Cho WY and Jo SK. Role of inflammation in the pathogenesis of cardiorenal syndrome in a rat myocardial infarction model. Nephrol Dial Transplant 2013; 28: 2766-2778.

[27] Han Z, Chen F, Ge X, Tan J, Lei P and Zhang J. miR-21 alleviated apoptosis of cortical neurons through promoting PTEN-Akt signaling pathway in vitro after experimental traumatic brain injury. Brain Res 2014; 1582: 12-20.

[28] Laatikainen LE, Incoronato M, Castellone MD, Laurila JP, Santoro M and Laukkanen MO. SOD3 decreases ischemic injury derived apoptosis through phosphorylation of Erk1/2, Akt, and FoxO3a. PLoS One 2011; 6: e24456.

[29] Zhou S, Zhang S, Wang Y, Yi S, Zhao L, Tang X, Yu B, Gu X and Ding F. MiR-21 and miR-222 inhibit apoptosis of adult dorsal root ganglion neurons by repressing TIMP3 following sciatic nerve injury. Neurosci Lett 2015; 586: 43-49.

[30] Obana M, Miyamoto K, Murasawa S, Iwakura T, Hayama A, Yamashita T, Shiragaki M, Kumagai S, Miyawaki A, Takewaki K, Matsumiya G, Maeda M, Yoshiyama M, Nakayama H and Fujio Y. Therapeutic administration of IL-11 exhibits the postconditioning effects against ischemia-reperfusion injury via STAT3 in the heart. Am J Physiol Heart Circ Physiol 2012; 303: H569-577.

[31] Rajasingh J, Bord E, Hamada H, Lambers E, Qin G, Losordo DW and Kishore R. STAT3-dependent mouse embryonic stem cell differentiation into cardiomyocytes: analysis of molecular signaling and therapeutic efficacy of cardiomyocyte precommitted mES transplantation in a mouse model of myocardial infarction. Circ Res 2007; 101: 910-918.

[32] Qiao S, Mao X, Wang Y, Lei S, Liu Y, Wang T, Wong GT, Cheung CW, Xia Z and Irwin MG. Remifentanil Preconditioning Reduces Postischemic Myocardial Infarction and Improves Left Ventricular Performance via Activation of the Janus Activated Kinase-2/Signal Transducers and Activators of Transcription-3 Signal Pathway and Subsequent Inhibition of Glycogen Synthase Kinase-3beta in Rats. Crit Care Med 2015; [Epub ahead of print].

[33] Haider KH, Idris NM, Kim HW, Ahmed RP, Shujia J and Ashraf M. MicroRNA-21 is a key determinant in IL-11/Stat3 anti-apoptotic signalling pathway in preconditioning of skeletal myoblasts. Cardiovasc Res 2010; 88: 168-178.