Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (2): 1-6
Published online May 2, 2015 (http://www.cellmolbiol.com)
Received on February 10, 2015, Accepted on April 12, 2015.
doi : 10.14715/cmb/2015.61.2.1

Cardioprotective effect of miR-214 in myocardial ischemic postconditioning by down-regulation of hypoxia inducible factor 1, alpha subunit inhibitor

D-Y. Wan[1], Z. Zhang[1] and H-H. Yang[2]✍

[1]Department of Cardiology, Zhengzhou People's Hospital, Zhengzhou, China
[2]Department of Cardiology, Henan Provincial People's Hospital, Zhengzhou, China

Corresponding author: Hong-hui Yang, Department of Cardiology, Henan Provincial People's Hospital, No.7 Weiwu Road, Jinshui District, 450003 Zhengzhou City, Henan Province, China. Email: honghyang@163.com

Abstract

To determine the significance of miR-214 expression in ischemic post-conditioning. Sixty rats were grouped to establish animal models. Immuno- luminescence and chemical methods were used to detect oxidative stress indicators. Hemodynamics indexes were measured by carotid artery intubation, and ischemia and infarction areas by Evans blue and 2,3-5 triphenyltetrazolium chloride(TTC) staining. TargetScan was used for identification and luciferase assays for verification of target genes.miR-214 and hypoxia inducible factor 1, alpha subunit inhibitor (HIF1AN) were analyzed by real-time quantitative polymerase chain reaction. Ischemia reperfusion significantly decreased left ventricular systolic pressure, +dp/dtmax, and -dp/dtmax and increased left ventricular end-diastolic pressure; ischemic post-conditioning had contrasting effects. Compared to the sham group, the ischemic/reperfusion (IR) group showed increased creatine kinase isoenzyme (CK-MB) and malondialdehyde (MDA) in the myocardium and decreased SOD. miR-214 in the IR group was down-regulated, and HIF1AN, up-regulated. Compared with the IR group, the ischemia postconditioning (IPC) group showed decreased CK-MB and MDA in the myocardium and increased SOD. The proportion of infarction area to ischemia area in IPC group declined compared to IR group. miR-214 and HIF1AN in the IPC group showed significant up- and down-regulation, respectively. Ischemic postconditioning can improve myocardial function, reduce myocardial infarction area, and prevent the ischemia reperfusion injury. miR-214 may participate in the protective function of ischemic post-conditioning by down-regulating HIF1AN.

Key words: miR-214, myocardium, ischemia reperfusion injury, ischemic postconditioning, HIF1AN.

Introduction

Coronary heart disease is a pathema with considerably high incidence and mortality, and is caused by myocardial ischemia, hypoxia or necrosis on account of vascular stenosis or occlusion arising from atherosclerosis of the coronary artery (1, 2). Currently, the most effective method for treatment is by reperfusion techniques such as use of thrombolytics, percutaneous coronary intervention (PCI) and vessel bypass grafting. All reperfusion techniques have potential to cause ischemia reperfusion injury (IRI) to the heart(3,4). Basic and clinical studies have proven that reperfusion arrhythmia, myocardial diastolic dysfunction, metabolic disorders, and change in myocardial ultra-structure may occur after myocardial ischemia reperfusion. Thus, IRI directly affects prognosis (5, 6).

In-depth study of reperfusion has shown that very early reperfusion is closely related to injuries, including changes in vascular endothelial cell function, systolic dysfunction, necrosis, and increase in apoptosis (7-9). Zhao et al. (10) first proposed the concept of ischemic postconditioning, which refers to alternating short bursts of re-occlusion and reperfusion within a short period after long-term myocardial ischemia, and then coronary arterial blood recovered totally. Some studies reported that ischemic postconditioning had a protective effect on the myocardium by relieving IRI and reducing the infarction area (11). Myocardial IRI produces a large amount of reactive oxygen species, which leads to structural damage and functional or metabolic disorders. Superoxide dismutase (SOD) is one of the important enzymes with antioxidant activity in the body, catalyzing the conversion of superoxide radicals generated in the process of metabolism to protect body tissues from damage. The activity of SOD indirectly reflects the body's capacity for scavenging free radicals and protection against oxidative damage (12). Creatine kinase (CK) is involved in oxidative phosphorylation and is sensitive to ischemia. When myocardial cells are damaged, increased levels and activity of CK act as indicators of severe myocardial injury (13). Malondialdehyde (MDA) is the main product of lipid peroxidation of poly unsaturated fatty acids in the myocardial cell membrane (14). Levels of CK, SOD and MDA can be used as parameters reflecting the degree of myocardial reperfusion injury. In addition, hemodynamic indicators reflect whether the cardiac function is normal, which is an important parameter for understanding the mechanism of ischemia-reperfusion injury (15).

miRNAs are small, non-coding single stranded RNAs, which inhibit the translation of mRNA by complementary base-pairing with the untranslated region at the 3'-end. Hence, miRNAs play a role in regulating the temporal expression of genes at the post-transcriptional level. miRNAs are involved in regulating cell differentiation, proliferation, and apoptosis and the immune response(16, 17). Due to tissue specific expression and stablility in circulation, an increasing number of miRNAs have become markers for early diagnosis and for monitoring recurrence and treatment (18-20). Studies in the cardiovascular field have reported that miRNAs play important roles in controlling cell differentiation, cell hypertrophy, hyperplasia, angiogenesis, endothe-

lial function, and ventricular remodeling (21). Abnormal expression of multiple miRNAs, including miRNA-195, miRNA-208, miRNA-133, and miRNA-21, regulates the process of ventricular remodeling (22-24). One study showed that miR-214 was up-regulated after rat myocardial infarction and speculated that miR-214 may be associated with ischemia and cell proliferation (25). miR-214 has also been shown to alter early cell differentiation, thereby influencing muscle cell development(26). Another study confirmed that miRNA-214 protects the mouse heart from ischemic injury by regulating Ca^{2+} overload and cell death (27). On the basis of bioinformatics prediction analysis, it has been estimated that miR-214 may regulate the expression of more than 300 target genes, including hypoxia inducible factor 1, alpha subunit inhibitor (HIF1AN). HIF1AN is a specific asparagine hydroxylase (28) that is an important inhibitor of hypoxia-inducible factor 1α (HIF-1α). HIF-1α is closely related to oxidative stress (29) and its up-regulation can significantly reduce cardiomyocyte apoptosis and enhance cardiac function (30). However, the role of miRNAs in myocardial ischemic post-conditioning is still unknown. Aurora et al. (27) reported that miRNA-214 protects the heart from ischemic injury by controlling Ca^{2+} overload and cell death. Here, we have discussed the significance of miRNA-214 expression in ischemic postconditioning.

Materials and methods

Experimental animals and grouping

Sixty Sprague-Dawley (SD) male rats weighing between 180 and 200g were provided by the experimental animal center of Zhengzhou University. They were randomly and equally divided into three groups: sham group, ischemic/reperfusion group (IR group), and ischemic postconditioning (IPC group).

Establishment of animal models

A compound anesthetic agent (0.1ml/30g) was used for inducing anaesthesia by intraperitoneal injection. Rats were fixed on the operating table and their limbs were linked with poles of the electrocardiograph machine to record the electrocardiogram (EGG). After insertion of the tracheal cannula, the ventilator started assisting in respiration with a breathing rate of 80/min and an inspiration to expiration ratio of 1:1.5. A skin incision was made left of the median line parallel to the sternum. Subcutaneous tissue and muscle were separated layer by layer. A chest expander was used between the third and fourth ribs to expose the heart. Between the left auricular appendage and the pulmonic stenosis area, a suture needle with 6-0 thread was passed through the ramus descendens anterior arteriae coronariae sinistrae. After ligation, the left ventricular wall showed cyanochroia or a light red color, and the wall motion weakened. The ST segment of the ECG was elevated, indicating that ST-segment elevation myocardial infarction had occurred. After ligation for 30min, the ligature was loosened and the cyanochroia in the ischemia area of the left ventricular anterior wall disappeared, followed by reperfusion recovery for 2h. For the IPC group, three alternating cycles of reperfusion and ischemia for 30s each was carried out at the time of reperfusion. All other treatments were the same as those for the IR group. For the sham group, the procedures were the same as those for the IR group, except for suture without ligation.

Detection of CK-MB, SOD, and MDA in the serum

The whole blood of rats was collected from a ventricle and the serum was stored. A chemiluminescence immunoassay (Beckman automatic instrument) was performed to detect the creatine kinase isoenzyme CK-MB and a chemical method was adopted to measure superoxide dismutase (SOD) and malondialdehyde (MDA).

Measurement of hemodynamics

The right carotid artery was exposed before open-heart surgery. The distal artery was ligated and the proximal portion was clamped with a bulldog clamp. A cardiac catheter was filled with normal saline (containing 625U/ml heparin) and was inserted into the left ventricle through intubation of the right carotid artery and linked to the biological signal collection and processing system through a baroreceptor. At the end of 2h of reperfusion, hemodynamics indexes such as left ventricular systolic pressure (LVSP), left ventricle end diastolic pressure (LVEDP), +dp/dtmax and -dp/dtmax were measured.

Detection of myocardial ischemia and necrotic area

After 2-h reperfusion, the heart was exposed through midline sternotomy. The arteria coronaria sinistra was ligated and blocked again. Evans blue (1%, 10ml) was injected through the left ventricular apex. When the hearts of rats were dyed blue, they were detached and washed with icy normal saline. The left ventricle were dissociated and kept in 1%TTC for 15min at 37°C. They were dried using filter paper and then kept at -20°C for 20min. The myocardial tissues were serially sectioned into 5 slices perpendicular to the long axis of the heart, with a thickness of approximately 2mm for per slice and fixed in 4% paraformaldehyde overnight. The infarction area was gray, non-infarction area was crimson and non-ischemia area was blue. The image analysis software Image J software was used to calculate the area of each part as well as the proportions of area at risk to left ventricular area (AAR/LV) and infarction area to area at risk (IS/AAR).

Selection and validation of miR-214 target genes

TargetScan was used to predict the target genes of miR-214 and luciferase assay was performed for validation. PCR amplification was performed for the target promoter fragment, HIF1AN-3'UTR, which was then inserted into the luciferase reporter gene plasmid PGL3-Basic to obtain PGL3-HIF1AN-3' UTR. Base mutations were introduced in the HIF1AN-3′UTR region and the resulting construct(5'-GUCACAUCCCCUGCUGGACGACA-3') was named PGL3-HIF1AN-3' UTR mut. The empty PGL3-Basic vector was used as negative control. The positive clones were screened and identified by sequencing, and then the corresponding plasmids were extracted. The transcription factor plasmid miR-214mimics was amplified and purified. H9C2 cells (rat cardiomyocytes; Biowit Technologies Ltd, Shenzhen, China) were cultured and seeded in a 24-well plate. After 10-24h (up to 80% confluence), the

reporter gene plasmids and transcription factor expression plasmids were co-transfected into H9C2 cells. Then, the luciferase activity was analyzed after addition of substrates.

Detection of miR-214 and HIF1AN by real-time quantitative polymerase chain reaction (RT- qPCR)

After 2-h reperfusion, 50mg myocardial tissues from the ischemic area were ground into powder in liquid nitrogen. Total miRNA was extracted using the miRcute miRNA Extraction and Isolation Kit (Beijing Tiangen Biotech, China) using 1ml lysate MZ. Poly (A) was added to the 3' end of miR-214 and reverse transcribed to synthesize the first chain of miRNA cDNA using the miRNA cDNA SS III kit(Beijing Tiangen Biotech, China) according to the manufacturer's instructions. RT-qPCR reaction was conducted using the miRcute miRNA fluorescent quantitative detection kit (Beijing Tiangen Biotech, China). The miR-214 primer was 5'-ACAGCAGGCACAGACAGGCAGT-3' and the primer for the reference gene 5s rRNA was 5'-GTCTACGGCCATACC ACCCTGAAC-3'.

Trizol was used to extract total mRNA, which was reverse transcribed to cDNA. Primers used for RT-qPCR of HIF1AN were 5'-GCCGCTACAACTAACCTAC-3' (forward primer) and5'-ATGGCAAGAG T CCAGTCC-3' (reverse primer). 18s rRNA was used as the housekeeping gene and detected using the primers5'-TACCACATCCAAGGAAGGCAGCA-3' (forward primer) and 5'-TGGAATTACCG AGGCTGCT-3' (reverse primer). All the primers were synthesized by Shanghai Sangon Biological Co., Ltd. Relative expression of the target gene was calculated in the terms of $2^{-\Delta\Delta Ct}$ values.

Statistical analysis

Matlab7.0 was used for data analyses. The different test parameters were compared among the three groups using the one-way ANOVA and t-test. The significant difference was defined as $P<0.05$.

Results

Changes of CK-MB, SOD and MDA in serum

The results (Table 1) showed that compared to the sham group, the IR group showed a distinct increase in the levels of CK-MB and MDA in the myocardium ($P<0.01$) and a remarkable decrease in SOD ($P<0.01$). Compared to the IR group, the IPC group showed a remarkable decrease in the levels of CK-MB and MDA in the myocardium ($P<0.05$) whereas the SOD levels were higher ($P<0.01$).

Comparative analysis of hemodynamics among the groups

Compared with the sham group, the IR and IPC groups showed a significant decrease in LVSP, +dp/dtmax, and -dp/dtmax and increase in LVEDP ($P<0.05$). Compared with the IR group, the IPC group showed an increase in LVSP, +dp/dtmax, and -dp/dtmax and significant decrease in LVEDP ($P<0.05$). There was no significant change in the heart rate among the three groups ($P>0.05$) (Table 2).

Changes in myocardial ischemia and infarction area

The IS/ AAR in the IPC group was lower than that in the IR group ($P<0.05$). The differences in AAR/LV for the IPC and IR groups were not significant ($P>0.05$) (Table 3).

Table 1. CK-MB, SOD and MDA Levels in Serum ($\bar{X}\pm s$)

Groups	CK-MB(IU/L)	SOD(nU/mL)	MDA(μmol/ml)
Sham group(n=20)	445.89±87.21	87.23±6.69	4.15±0.55
IR group(n=20)	1978.12±300.91**	45.75±9.68**	6.49±1.35*
IPC group(n=20)	1047.02±145.36**##	69.77±9.37**##	2.12±0.55**##

*P<0.05, **P<0.01 vs sham group; ##P<0.01 vs IR group.

Table 2. Hemodynamics Data of Rats in Each Group ($\bar{X}\pm s$)

Groups	LVSP (mmHg)	LVEDP (mmHg)	+dp/dt max(mmHg/s)	-dp/dt max(mmHg/s)	HR (times/min)
Sham group(n=20)	135.47±10.24	-8.11±2.12	8524.8±541.55	-6247±603.41	436±32
IR group(n=20)	95.34±7.98*	6.07±3.78*	4223.9±478.41*	-3467±184.26*	363±54
IPC group(n=20)	110.70±7.04*#	-1.53±0.91*#	5724.6±427.67*#	-4427±287.45*#	388±24

*P<0.05 vs sham group; #P<0.05 vs IR group.

Table 3. Comparison of Data for Myocardial Ischemia and Infarction Area ($\bar{X}\pm s$)

Groups	IS/ AAR (%)	AAR/LV (%)
IR group	50.17±4.12	24.66±10.46
IPC group	31.55±4.08*	26.87±6.49

*P<0.05 vs IR group.

Validation of target gene for miR-214

Bioinformatics analysis showed that seven complementary base pairs were located in the binding position of miR-214 and the HIF1AN-3' UTR. The fluorescent plasmid reporting system verified that HIF1AN was the target gene of miR-214. miR-214 mimics could inhibit the luciferase activity of the HIF1AN-3' UTR vector by up to 45% (P<0.01), (Figure 1).

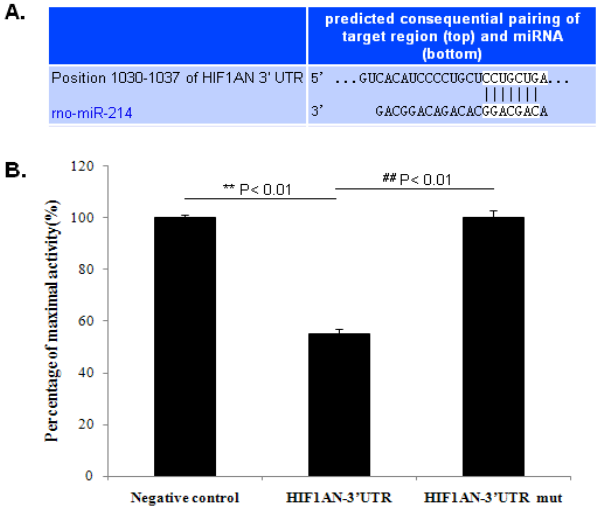

Figure 1. Prediction and validation of the target gene. A. Binding site of miR-214 in the HIF1AN -3' UTR; B. Validation of HIF1AN as the target gene of miR-214 by a luciferase activity assay. Empty PGL3-basic vector was used as the negative control. PGL3-Basic vector containing the HIF1AN-3′UTR fragment is shown as HIF1AN-3′UTR and PGL3-basic vector containing HIF1AN-3′UTR mutant is shown as HIF1AN-3′UTR mut. **P<0.01 vs negative control; $^{\#\#}$P<0.01 vs HIF1AN-3′UTR.

Expression of miR-214 and HIF1AN

As shown in Figure 2, miR-214 expression in the IR group was downregulated (P<0.01), wherea the expression of HIF1AN was upregulated (P<0.05) compared to that in the sham group. Compared to the IR group, the IPC group showed miR-214 upregulation (P<0.01) and remarkable HIF1AN downregulation (P<0.05). However, no significant difference was detected between the IPC and sham groups (P>0.05).

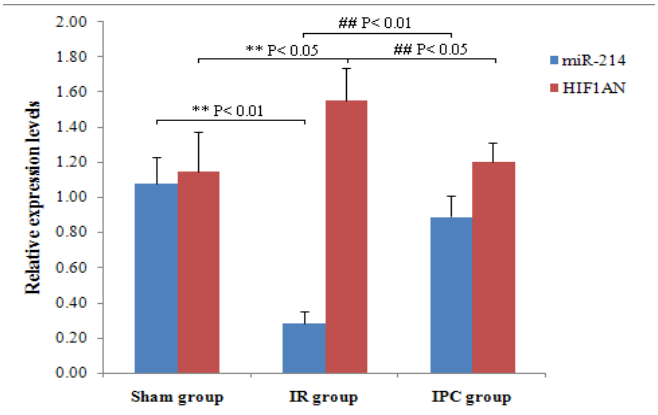

Figure 2. Changes in the expression of miR-214 and HIF1AN. *P<0.05, **P<0.01 vs sham group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs IR group.

Discussion

Myocardial IRI produces a large number of oxygen free radicals that trigger lipid chain peroxidation, damage the membrane system, promote cellular oxidative damage and change the membrane permeability and fluidity. Furthermore, they can attack proteins, leading to loss of or decrease in enzymatic activity (31, 32). CK mainly exists in the skeletal muscle, myocardium and brain tissues. The CK-MB subtype exists in myocardial tissues in which few other subtype CKs appear. Therefore, CK-MB is regarded as the main marker to measure the degree of injury to the myocardium (13). MDA is the marker used to assess the severity of membrane lipid peroxidation damage. SOD, as a scavenger of free radicals, catalyzes the conversion of free radicals such as superoxide anions by the disproportionation reaction to protect the organism (12). This study considered CK-MB, SOD and MDA as the indexes for evaluating the modeling of myocardial ischemia reperfusion. The results showed that, compared with the sham group, the IR group showed significantly increased CK-MB and MDA and decreased SOD levels. Compared to the IR group, the IPC group showed decreased CK-MB and MDA and increased SOD, which indicated that ischemic postconditioning could protect the organism from injury.

The enhanced ability of the myocardium to resist long-term severe ischemia reperfusion after suffering one or more repetitive and transitory ischemia reperfusions was first discovered and named ischemic preconditioning by Murry et al. (33). Halkos et al. (34, 35) compared post-conditioning with ischemic preconditioning, which was famous as a gold standard for cardiac endogenous protection, and found that the reduced infarction area was similar between ischemia preconditioning and postconditioning. Further research by Zhao et al. (10) demonstrated that ischemic postconditioning could limit the infarction area and lessen tissue edema in the ischemic myocardium and neutrophils accumulation, while improving the function of endothelial cells. Our study showed that ischemic postconditioning could remarkably increase LVSP, +dp/dtmax and -dp/dtmax and decrease LVEDP, improve cardiac function and reduce myocardial infarction area. This was in accordance with related conclusions that ischemia postconditioning had a protective effect on the heart (36). However, the mechanism underlying such a cardioprotective effect is still unclear.

Specific miRNA can indicate the severity of disease to some extent (37, 38). Our study suggested that miR-214 may participate in the process of protecting the myocardium after ischemic post-conditioning. The RT-qPCR results showed that the expression of miR-214 in severely injured tissues was low and that it increased after ischemic post-conditioning. HIF1AN, one of the target genes of miR-214, showed higher expression in the IR group than sham group. Lowering the activity of HIF1AN can enhance the activity of HIF-1α (39, 40). However, the expression of HIF1AN remarkably decreased in the IPC group. This indicated that the downregulation of HIF1AN could protect cardiac cells. In conclusion, it was inferred that miR-214 may participate in cardiac protection in the case of treatment with

myocardial ischemic postconditioning, through inhibition of HIF1AN expression.

Acknowledgements

This study was supported by grants from the Henan Province Zhengzhou City Science and Technology Plan Project (NO. 083SGYS33238_2).

References

1. Kim, AS., and Johnston, S.C., Global variation in the relative burden of stroke and ischemic heart disease. *Circulation*. 2011, **124**: 314-23.doi: 10.1161/ CIRCULATI ONAHA.111.018820

2. Greenwood, J.P, Maredia, N., Younger, J.F., Brown, J.M., Nixon, J., Everett, C.C., Bijsterveld, P., Ridgway, J.P., Radjenovic, A., Dickinson, C.J., Ball, S.G.and Plein, S., Cardiovascular magnetic resonance and single-photon emission computed tomography for diagnosis of coronary heart disease (CE-MARC): a prospective trial. *Lancet*. 2012, **379**: 453-60. doi: 10.1016/S0140-6736(11)61335-4

3. Cohen, D.J., Van Hout .B., Serruys, P.W., Mohr, F.W., Macaya, C., den Heijer, P., Vrakking, M.M., Wang, K., Mahoney, E.M., Audi, S., Leadley, K., Dawkins,K.D. and Kappetein,A.P., Quality of life after PCI with drug-eluting stents or coronary-artery bypass surgery. *N Engl J Med*. 2011, **364**: 1016-26. doi: 10.1056/NEJMoa1001508

4. Brilakis, E.S., Rao, S.V., Banerjee, S., Goldman, S., Shunk,K,A., Holmes DR, Jr,, Honeycutt, E. and Roe, M.T., Percutaneous coronary intervention in native arteries versus bypass grafts in prior coronary artery bypass grafting patients: a report from the National Cardiovascular Data Registry. *JACC Cardiovasc Interv*. 2011, **4**: 844-50.

5. Becker, L.C. and Ambrosio. G., Myocardial consequences of reperfusion. *Prog Cardiovasc Dis.* 1987, **30**: 23-44. doi: 10.1016/0033-0620(87)90009-0

6. Matsumura, K., Jeremy, R.W., Schaper, J. and Becker, L.C., Progression of myocardial necrosis during reperfusion of ischemic myocardium. *Circulation*. 1998, **97**: 795-804. doi: 10.1161/01.CIR.97.8.795

7. Baxter, G.F., The neutrophil as a mediator of myocardial ischemia-reperfusion injury: time to move on. *Basic Res Cardiol.* 2002, **97**: 268-75. doi: 10.1007/s00395- 002-0366-7

8. Baxter, G.F. and Yellon, D.M., Current trends and controversies in ischemia-reperfusion research--meeting report of the Hatter Institute 3rd International Workshop on Cardioprotection. *Basic Res Cardiol.* 2003, **98**: 133-6.

9. Bolli, R., Becker, L., Gross, G., Mentzer R, Jr., Balshaw, D. and Lathrop, D.A., Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ Res*. 2004, **95**: 125-34. doi: 10.1161/01.RES.0000137171.97172.d7

10. Zhao, Z.Q., Corvera, J.S., Halkos, M.E., Kerendi, F., Wang, N.P., Guyton, R.A. and Vinten-Johansen, J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol.*2003, **285**: H579-88.

11. Kin, H., Zhao, Z.Q., Sun, H.Y., Wang, N.P., Corvera, J.S., Halkos, M.E., Kerendi, F., Guyton,R.A. and Vinten-Johansen ,J. Postconditioning attenuates myocardial ischemia–reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardiovasc Res*. 2004, **62**: 74-85. doi: 10.1016/j.cardiores.2004.01.006

12. Zhou, Q., P457Effect of changed oxygen free radical on cardiac function of plateau migrants after returning to the low altitude. *Cardiovas Research*. 2014, **103**: S84-S.

13. Cavallini, C., Savonitto, S., Violini, R., Arraiz, G., Plebani, M., Olivari, Z., Rubartelli, P., Battaglia, S., Niccoli, L., Steffenino, G., Ardissino, D. Italian 'Atherosclerosis, Thorombosis, and Vascular Biology' and 'Society for Invasive Cardiology-GISE' Investigators. Impact of the elevation of biochemical markers of myocardial damage on long-term mortality after percutaneous coronary intervention: results of the CK-MB and PCI study. *Eur Heart J.* 2005, **26**: 1494-8.

14. Guler, L., Bozkirli, F., Bedirli, N., Unal, Y., Guler, A., Oztas,Y., Balta, S., Cakar, M., Demirkol, S., Arslan, Z, and Unlu, M. Comparison of the Effects of Dexmedetomidine vs. Ketamine in Cardiac Ischemia/Reperfusion Injury in Rats - Preliminary Study. *Adv Clin Exp Med.* 2014, **23**:683-9.doi: 10.17219/acem/37214

15. Liu, H., Guo, X., Chu, Y., and Lu, S. Heart protective effects and mechanism of quercetin preconditioning on anti-myocardial ischemia reperfusion (IR) injuries in rats. *Gene*. 2014, **15**;545:149-55.doi: 10.1016/j.gene.2014.04.043

16. Chen, F. and Hu, S.J. Effect of microRNA-34a in cell cycle, differentiation, and apoptosis: A review. *J Biochem Mol Toxicol.* 2012, **26**: 79-86. doi: 10.1002/jbt.20412

17. Curtale, G., Citarella, F., Carissimi, C., Goldoni, M., Carucci, N., Fulci, V., Franceschini, D., Meloni, F., Barnaba. V.and Macino, G. An emerging player in the adaptive immune response: microRNA-146a is a modulator of IL-2 expression and activation-induced cell death in T lymphocytes. *Blood*. 2010, **115**: 265-73. doi: 10.1182/blood-2009-06-225987

18. Li, L., Chen, X.P. and Li, Y,J., MicroRNA-146a and Human Disease. *Scand J Immunol*. 2010, **71**: 227-31. doi: 10.1111/j.1365-3083.2010.02383.x

19. Borel, F., Konstantinova, P. and Jansen, P.L., Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. *J Hepatol.* 2012, **56**: 1371-83. doi: 10.1016/j.jhep.2011.11.026

20. Lu, T.X. and Rothenberg, M.E., Diagnostic, functional, and therapeutic roles of microRNA in allergic diseases. *J Allergy Clin Immunol.* 2013, **132**: 3-13. doi: 10.1016/j.jaci.2013.04.039

21. Meloni, M., Marchetti, M., Garner, K., Littlejohns, B., Sala-Newby, G., Xenophontos, N., Floris, I., Suleiman, M.S., Madeddu, P., Caporali, A.and Emanueli, C., Local inhibition of microRNA-24 improves reparative angiogenesis and left ventricle remodeling and function in mice with myocardial infarction. *Mol Ther*. 2013,**21**:1390-402. doi: 10.1038/mt.2013.89

22. Graham, R.M. and Harvey, R.P., The ontogeny of cardiac regeneration. *Circ Res*. 2011, **108:** 1304-5. doi: 10.1161/RES.0b013e318222ba1a

23. Prado-Uribe, M.D., Soto-Abraham, M.V., Mora-Villalpando, C.J., Gallardo, J.M., Bonilla, E., Avila, M., Tena, E.and Paniagua, R., Role of thyroid hormones and mir-208 in myocardial remodeling in 5/6 nephrectomized rats. *Arch Med Res.* 2013, **44:** 616-22.

24. Xu, G., Gan, T., Tang, B., Chen, Z.H., Ailiman, M., Zhou, X.H., Jiang, T., Song, J.G., Guo, X., Li ,Y.D., Miao, H.J., Zhang ,Y.and Li, J.X., Changes in microRNAs expression are involved in age-related atrial structural remodeling and atrial fibrillation. *Chin Med J (Engl).* 2013, **126**: 1458-63.

25. Shi, B., Guo, Y., Wang, J., and Gao, W. Altered expression of microRNAs in the myocardium of rats with acute myocardial infarction. BMC Cardiovasc Disord. 2010, **1**;10:11.doi: 10.1186/1471-2261-10-11

26. Flynt, A.S., Li, N.,Thatcher, E.J., Solnica-Krezel, L., and Patton, J.G. Zebrafish miR-214 modulates Hedgehog signaling to specify muscle cell fate. *Nat Genet*. 2007. **39**:259–263.doi: 10.1038/ng1953

27. Aurora ,A.B, Mahmoud, A.I., Luo, X., Johnson, B.A., van Rooij, E, Matsuzaki, S., Humphries, K.M., Hill, J.A., Bassel-Duby, R., Sadek,H.A. and Olson, E.N. MicroRNA-214 protects the mouse heart from ischemic injury by controlling Ca(2)(+) overload and cell death. *J Clin Invest.* 2012,**122**: 1222-32.

28. Lee, C., Kim, S.J., Jeong, D.G., Lee, S.M. and Ryu, S.E. Struc-

ture of human FIH-1 reveals a unique active site pocket and interaction sites for HIF-1 and von Hippel-Lindau. J Biol Chem. 2003, **278**: 7558-63. doi: 10.1074/jbc.M210385200

29. Kim, C,H., Cho ,Y.S., Chun, Y.S., Park, J.W. and Kim, M.S. Early expression of myocardial HIF-1alpha in response to mechanical stresses: regulation by stretch-activated channels and the phosphatidylinositol 3-kinase signaling pathway. *Circ Res*. 2002, **90**: E25-33. doi: 10.1161/hh0202.104923

30. Poynter, J.A., Manukyan, M.C., Wang, Y., Brewster, B.D., Herrmann, J.L., Weil, B.R., Abarbanell, A.M. and Meldrum, D.R. Systemic pretreatment with dimethyloxalylglycine increases myocardial HIF-1alpha and VEGF production and improves functional recovery after acute ischemia/reperfusion. *Surgery*. 2011, **150**: 278-83. doi: 10.1016/j.surg.2011.06.006

31. Patel ,Y., Collaco Moraes, Y., Latchman, D., Coffin ,R. and de Belleroche, J., Neuroprotective effects of copper/zinc-dependent superoxide dismutase against a wide variety of death-inducing stimuli and proapoptotic effect of familial amyotrophic lateral sclerosis mutations. *Brain Res Mol Brain Res.* 2002, **109**: 189-97. doi: 10.1016/S0169-328X(02)00560-0

32. Volk, T,, Schmutzler, M., Engelhardt, L., Pantke, U., Laule, M., Stangl, K., Grune, T., Wernecke, K.D., Konertz, W. and Kox, W.J. Effects of different steroid treatment on reperfusion-associated production of reactive oxygen species and arrhythmias during coronary surgery. *Acta Anaesthesiol Scand.* 2003, **47**: 667-74.

33.Murry, C.E., Jennings, R.B. and Reimer, K.A., Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986, **74**: 1124-36. doi: 10.1161/01.CIR.74.5.1124

34. Halkos, M.E., Kerendi, F., Corvera, J.S, Wang, N.P., Kin, H., Payne, C.S., Sun, H.Y., Guyton, R.A., Vinten-Johansen, J. and Zhao, Z.Q. Myocardial protection with postconditioning is not enhanced by ischemic preconditioning. *Ann Thorac Surg.* 2004, **78**: 961-9. doi: 10.1016/j.athoracsur.2004.03.033

35. Ma, X., Weyrich, A.S., Lefer, D.J. and Lefer, A.M., Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. *Circ Res.* 1993, **72**: 403-12. doi: 10.1161/01.RES.72.2.403

36. Vinten-Johansen, J., Zhao, Z.Q., Zatta,A.J, Kin, H., Halkos, M.E. and Kerendi, F. Postconditioning A new link in nature's armor against myocardial ischemia–reperfusion injury. *Basic Res Cardiol.* 2005, **100**: 295-310.

37.Papagregoriou, G., Erguler, K., Dweep, H., Voskarides, K., Koupepidou, P., Athanasiou ,Y., Pierides, A., Gretz, N., Felekkis, K.N. and Deltas,C. A miR-1207-5p binding site polymorphism abolishes regulation of HBEGF and is associated with disease severity in CFHR5 nephropathy. *PloS one*. 2012, **7**: e31021. doi: 10.1371/journal.pone.0031021

38. Castro, R.E., Ferreira, D., Afonso, M.B., Borralho, P.M., Machado, M.V., Cortez-Pinto, H.and Rodrigues, C.M. miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. *J Hepatol*. 2013, **58**: 119-25. doi: 10.1016/j.jhep. 2012.08.008

39. Mahon, P.C, Hirota, K. and Semenza, G.L. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev.* 2001, **15**: 2675-86. doi: 10.1101/gad.924501

40. Lando, D., Peet, D.J., Whelan, D.A., Gorman, J.J. and Whitelaw, M.L. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* . 2002, **295**: 858-61. doi: 10.1126/science.1068592