Int J Clin Exp Med 2015;8(9):15514-15520
www.ijcem.com /ISSN:1940-5901/IJCEM0006642

*Original Article*

# Effect of Si-RNA-silenced HIF-1α gene on myocardial ischemia-reperfusion-induced insulin resistance

Feng Wang, Gui-You Liang, Da-Xing Liu, Quan Tang, Jian Zhang, Qing-Yong Cai, Deng-Shen Zhang, Xu Han

*Department of Thoracic and Cardiovascular Surgery, Affiliated Hospital of Zunyi Medical College, Zunyi 563003, P. R. China*

Received February 3, 2015; Accepted April 3, 2015; Epub September 15, 2015; Published September 30, 2015

**Abstract**: Objective: To explore the effect (expression and implication) of hypoxia-inducible factor-1α (HIF-1α) silence induced by siRNA on the myocardial ischemia-reperfusion-induced insulin resistance in adult rats. Methods: One-step enzymolysis method was used to isolate adult rat cardiomyocytes; adult rat cardiomyocytes were cultured; HIF-1α gene-specific Si-RNA was constructed and transfected into rat cardiomyocytes using liposome method. Myocardial IRI model was prepared. HIF-1α and glucose transporter 4 (GLUT-4) mRNA expression was detected by RT-PCR; distribution of GLUT-4 protein expression in adult rat cardiomyocytes was detected by immunofluorescence; Western blot was used for the detection of HIF-1αprotein expression; isotope tracer assay was used to detect the changes in cell glucose (Glu) uptake rate. Results: This method can stably get 85% to 90% active calcium tolerant adult rat cardiac myocytes, and the cultured cells were proved to be cardiomyocytes. After experiencing ischemia-reperfusion injury, HIF-1α mRNA expression levels in adult rat hypoxia cardiomyocytes had different degrees of increase compared with the control group (compared with the control group, $P < 0.05$). Compared with the model group, HIF-1α mRNA expression levels after ischemia and reperfusion in HIF-1αsi-RNA group and empty-vector group were lower than that in the control group and the model group; the expression reached the peak after 60 min of reperfusion, which did not change significantly in the control group. Expression of HIF-1α protein in myocardial cells was quite low in the control group; in the model group and intervention group, only after hypoxia-ischemia for 60 min, expression bands could be detected; especially in the model group, the expression had been increased until 60 min after reperfusion and began to decline from the time point of 180 min after reperfusion, but was still higher than that in the control group; in the intervention and empty-vector groups, it also increased rapidly at 60 min, but the expression was significantly lower than that in the model group; at 180 min after reperfusion, its protein expression peaked; while at 8 h after reperfusion, all the expression was extremely low. Compared with the control group, Glut4 mRNA expression in model group, transfected group and empty-vector group was reduced at the time points of T1-T4 ($P < 0.05$); the decline was the most significant at the time points of T1 and T2, followed by slightly increase at T3 and gradual recovery at T4; Compared with model group, Glut4 mRNA expression in transfection group was significantly reduced ($P < 0.05$); the decline was the most obvious at T1-T2, and then there was an increasing trend and it was recovered at T5 point. After experiencing ischemia, GLUT-4 protein expression changing trend was as follows: it was significantly reduced on the cell membrane, which was the most obvious from T1 to T3 and began to improve at T3, but still had not reached the level in the control group; it had been reached the levels of the control group at T5. After HIF-1αsi-RNA transfection and ischemia, GLUT-4 protein expression was increased in plasma and reduced on cell membrane; the decline was slightly improved at T3 and recovered to control distribution level at T5. After cardiac ischemia-reperfusion, glucose uptake rate decreased to varying degrees in myocardial cells and reached the lowest value after 60 min of ischemia, then gradually increased. After 8 h of reperfusion, the level in model group returned to the control level; compared with the model group, glucose concentration increased more serious in transfection group and empty-vector group after reperfusion. Conclusion: HIF-1α played a central regulatory role in this mechanism; HIF-1α may be one of the molecular mechanisms triggering myocardial IR.

**Keywords:** Adult rat cardiomyocytes, ischemia-reperfusion, insulin resistance, hypoxia-inducible factor-1α, transfection

## Introduciton

Ischemia and reperfusion (IR) injury (IRI) is a primary cause of cardiac failure, morbidity, mortality after cardiac operations [1] or heart infarctions [2]. Over the past decade, the transcriptional complex hypoxia inducible factor-1α (HIF-1α) has emerged as a key regulator of the mole-

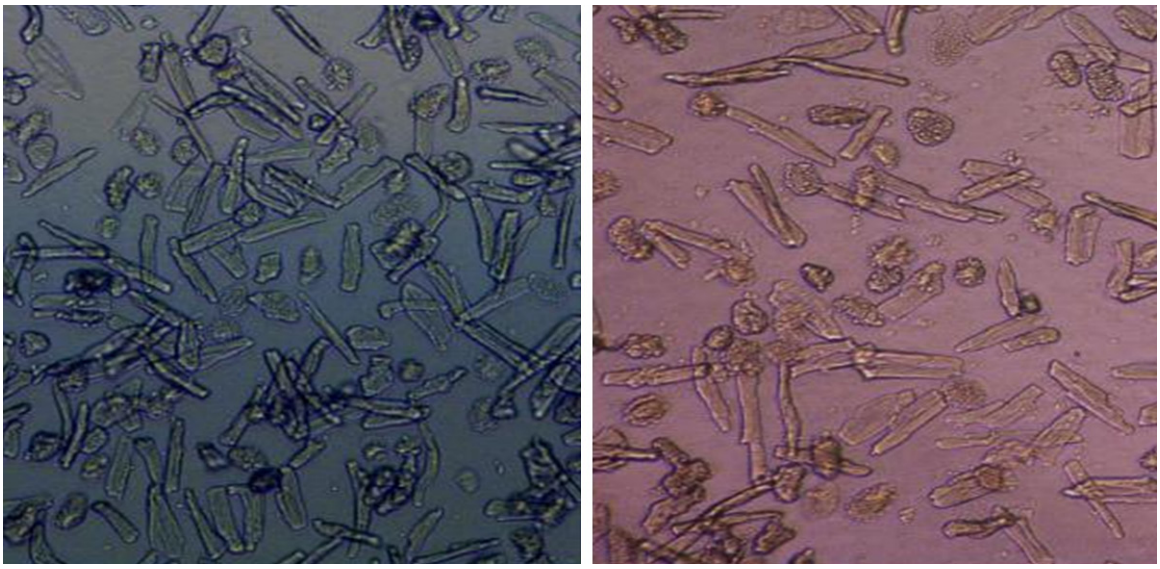

**Figure 1.** The isolated and non-adherent myocardial cells; Single adult rat ventricular myocyte was rod-shaped or rectangular-shaped, with clear stripes and clear angular; a small number of cardiomyocytes had spontaneous rhythmic Shrink (× 100).

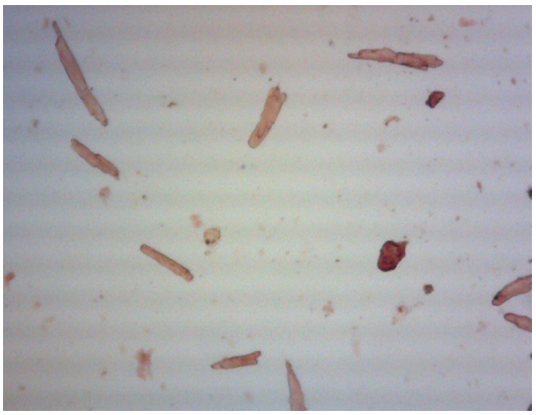

**Figure 2.** Cardiomyocytes immunohistochemical images; after DAB staining, myocardial intracellular actin was stained brown, which confirmed that they were cardiomyocytes (× 200).

cular hypoxic response and is a master regulator of the cellular and systemic homeostatic responses to hypoxia by activating the transcription of many genes [3-7], including those involved in energy metabolism, angiogenesis, apoptosis, and other genes, the protein products of which increase oxygen delivery or facilitate metabolic adaptation to hypoxia [8]. HIF-1α plays an essential role in embryonic vascularization, tumor angiogenesis, and the pathophysiology of ischemic disease. In particularly, HIF-1α activation plays an essential role in triggering cellular protection and metabolic alterations in response to oxygen deprivation during myocardial ischemia [9].

In this study, the adult rat model of cardiac ischemia-reperfusion-induced insulin resistance was established and HIF-1α mRNA expression and protein levels in myocardial cells in the process of IR were detected to observe the effect of HIF-1α silence on insulin resistance (IR) and the protective effect of its target gene expression in ischemia-reperfusion injury (IRI) process and the possible mechanism, in order to explore the key role of HIF1-α in IRI IR formation, thus to find effective protection measures for cardiopulmonary bypass to block myocardial IR, prevent the occurrence and development of cardiac IRI, promote the recovery of postoperative cardiac function and reduce mortality in patients after cardiopulmonary bypass surgery.

## Material and methods

### *Experimental animals and grouping*

In this study, 200 adult SD rats were selected, aged 18 to 20 weeks, weighting between 200 and 250 g, male or female. After langendorff cardiac perfusion, cardiomyocytes were isolated using one-step enzymatic digestion method for cell culture; When the culture was stable,

**Table 1.** HIF-1α mRNA expression detection after ischemia-reperfusion injury ($\bar{x}$±s)

| Groups | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| Control group | 24.71±0.99 | 24.77±1.76 | 27.95±1.17 | 26.57±1.33 | 26.40±1.13 |
| Model group | 249.03±4.21* | 256.46±6.24* | 271.31±3.01* | 247. 61±3.83* | 30.76±1.59 |
| Empty-vector group | 189.03±3.21* | 199.46±5.14* | 221.31±2.91* | 136. 61±2.83* | 31.76±1.29 |
| Transfection group | 185.70±1.56*,#,& | 193.12±3.51*,#,& | 224.65±2.87*,#,& | 132.94±2.82*,#,& | 29.09±1.06 |

Note: Compared with control group,*$P$ < 0.05. Compared with model group, #$P$ < 0.05; Compared with empty-vector group, &$P$ < 0.05.

**Table 2.** Ratios of HIF-1α and internal control bands after myocardial ischemia/reperfusion injury ($\bar{x}$ ±s)

| Groups | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| Control group | 0.11±0.03 | 0.08±0.02 | 0.03±0.02 | 0.11±0.04 | 0.06±0.02 |
| Model group | 3.83±0.29* | 4.57±0.24* | 5.72±0.35* | 4.35±0.20* | 3.51±0.24* |
| Empty-vector group | 2.16±0.51* | 3.01±0.22* | 3.37±0.13* | 2.64±0.19* | 2.46±0.18* |
| Transfection group | 2.26±0.61*,# | 3.06±0.25*,# | 3.57±0.14*,# | 2.74±0.21*,# | 2.56±0.24*,# |

Note: Compared with control group, *$P$ < 0.05; Compared with model group, #$P$ < 0.05; Compared with empty-vector group, &$P$ < 0.05.

cells were divided into four groups based on interventions: the control group, without hypoxia-reoxygenation, cultured for 13 hours; the model group; empty-vector transfected group (transfected with unrelated sequences) and HIF-1α si-RNA intervention group; the later three groups experienced hypoxia for 60 min, reoxygenation for 15 min, 60 min, 180 min and 8 h (T1-T5), and corresponding specimens were collected at T1-T5 time points. Experimental animals were provided by the Experimental Animal Center of Third Military Medical University.

*Instruments and reagents*

Real Time PCR kit and the design and synthesis of HIF-1α receptor primer were provided by Dalian Takara Biotechnology Co.; HIF-1αsi-RNA design, synthesis and screening were completed by Guangzhou RuiBo biological reagent company; the rest reagents were analytical grade or biochemical grade.

*Methods*

Adult rat cardiomyocytes were isolated using one-step enzymatic digestion method for cell culture; HIF-1α gene-specific Si-RNA was constructed and transfected into rat cardiomyocytes using liposome method. Myocardial IRI model was prepared. HIF-1α and glucose transporter 4 (GLUT-4) mRNA expression was detected by RT-PCR; distribution of GLUT-4 protein expression in adult rat cardiomyocytes was detected by immunofluorescence; Western blot was used for the detection of HIF-1αprotein expression; isotope tracer assay was used to detect the changes in Glu uptake rate.

*Statistical analysis*

Data were expressed as mean ± standard deviation ($\bar{x}$±s), using SPSS19.0 software for analysis. Averages at each time point were compared using ANOVA: homogeneity of variance was tested by LSD, with Games-Howell method when variance missing. $P$ < 0.05 was considered statistically significant.

## Results

*Isolation, culture and identification of adult rat cardiomyocytes*

This method can stably obtain 85% to 90% active calcium tolerant adult rat cardiomyocytes (**Figure 1**). After DAB staining, cytoplasm was stained brown (**Figure 2**); after the hematoxylin re-staining, muti-nuclei can be observed and stained blue violet, which confirmed that the isolated cells were cardiomyocytes.

*Changes in HIF-1α mRNA level*

After experiencing ischemia-reperfusion injury, expression of HIF-1α mRNA in adult rat cardiomyocytes were increased to varying degrees in

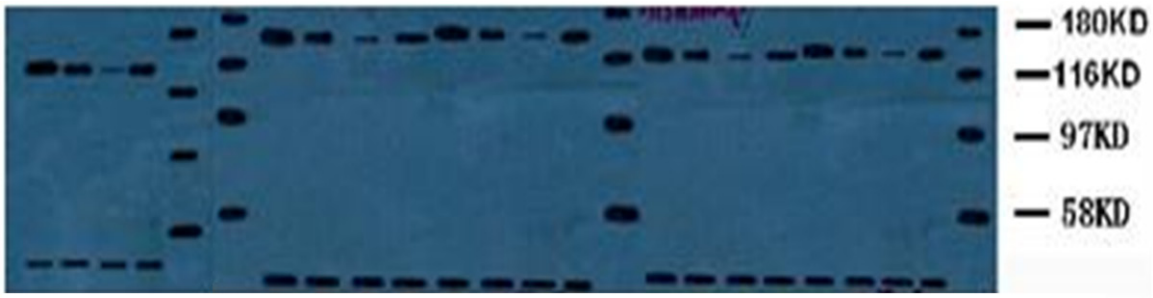


**Figure 3.** From left to right, both sides were Maker; the first row was the internal control β-tubulin; 2nd to 4th rows were respectively the T1 bands in model group, intervention group, the control group and empty-vector group, sequentially arranging until T5 time point; there was basically no expression in the control group, and the expression level was very low; expression was significantly increased in model group, followed by transfection group.

**Table 3.** Expression of GLUT-4 mRNA in adult rat cardiac ischemia and reperfusion ($\bar{x}\pm s$)

| | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| Control group | 107.75±2.40 | 108.56±1.51 | 108.96±3.36 | 108.54±2.66 | 107.80±1.70 |
| Model group | 85.99±2.14* | 93.03±2.76* | 96.76±2.66* | 104.15±0.85 | 109.13±2.19 |
| Empty-vector group | 70.48±2.55* | 80.54±2.65* | 88±1.33* | 96.23±2.63* | 105.91±3.58 |
| Transfection group | 72.48±3.05*,# | 82.84±3.25*,# | 92±1.53*,# | 99.33±2.83*,# | 107.91±4.08 |

Note: Compared with control group, *$P < 0.05$; Compared with model group, #$P < 0.05$.

model group compared with the control group (compared with the control group, $P < 0.05$). Compared with the model group, the expression of HIF-1α mRNA after ischemia and reperfusion in HIF-1αsi-RNA group was lower than that in the control group and the model group, but it had its own changing trend; at 60 min after re-perfusion, its expression level reached the peak, and then began to decline at 180 min, consistent with the trend of model group (peaked at 60 min after reperfusion); expression level did not change significantly in the control group, and the difference was not statistically significant within the group, shown in **Table 1**.

*Changes in HIF-1α expression*

Image analysis for the bands of Western blot showed that: The expression of HIF-1α protein in myocardial cells of the control group was very low; in the model group and intervention group, the bands could be detected after 60 min of hypoxia-ischemia; particularly in the model group, the expression was increased from beginning to 60 min of reperfusion, and began to decline at 180 min after reperfusion, but it was still higher than that in the control group; The expression in intervention group was also rapidly increasing at 60 min after hypoxia-ischemia, but the expression was significantly lower than that in the model group; at 180 min after reperfusion, the protein expression peaked; at 12 h after reperfusion, we can see that in the control group, model group and the intervention group, the expression was extremely low, even cannot be detected (**Table 2**; **Figure 3**).

*Expression and translocation of GLUT-4 in adult rat cardiac ischemia and reperfusion*

Glut4mRNA expression: control group showed no significant difference in expression at T1-T5 ($P > 0.05$); compared with the control group, the expression was reduced at T1-T4 in model group, transfection group, and empty-vector group ($P < 0.05$); At T1 and T2, the expression decreased most significantly, and then slightly increased, gradually restored at T4; compared with the model group, Glut4 mRNA expression was significantly reduced in transfection group ($P < 0.05$); the expression decreased most significantly between T0 and T2, then showed an increasing tendency, and recovered at T5, shown in **Table 1**; as shown in the picture, we can see that at each time point in the control group, GLUT-4 evenly distributed in the cytoplasm and cell membrane, without differences; after experiencing ischemia, the cytoplasm GLUT-4 protein expression changing trend was as follows: in the cell membrane, the expres-

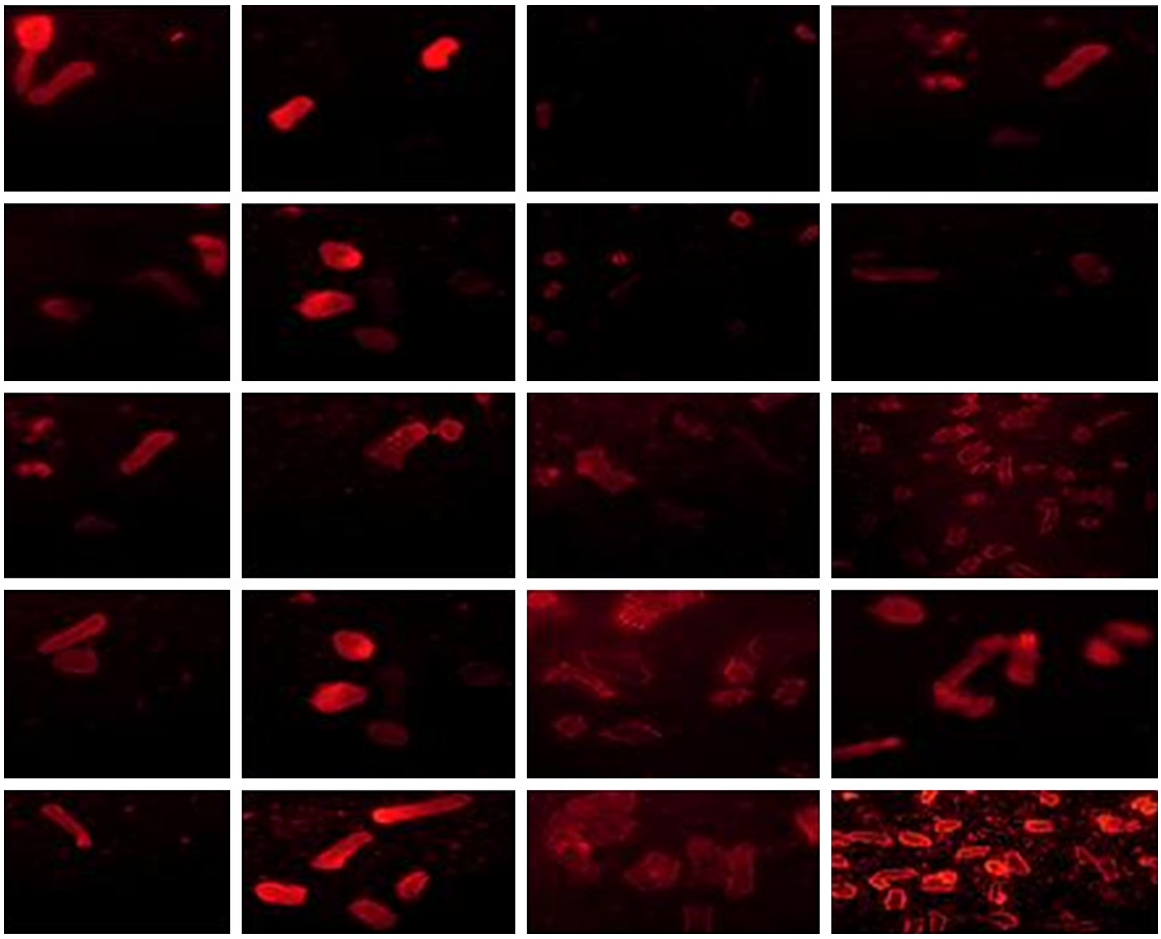

**Figure 4.** Fluorescent Photo: From the left to the right, they respectively were the pictures for the distribution of GLUT-4 in the cytoplasm and cell membrane in the control group, model group, empty-vector group and Si-RNA transfection group at each time point; after ischemia, cytoplasm GLUT- 4 more protein was increased, and there was a more pronounced reduction in the cell membrane, which was slightly improved at T3 and returned to the control level at T5.

sion was significantly reduced, which decreased most significantly at T1, at T3 began to improve but still had not reached the level of the control group, and returned to distribution level of the control group at T5. After HIF-1αsi-RNA transfection and ischemia, cytoplasm GLUT-4 protein expression was increased, but it was significantly reduced in cell membrane, which was slightly improved at T3 and returned to the control level at T5 (**Table 3**; **Figure 4**).

*Adult rat myocardial glucose uptake rate*

After myocardial ischemia and reperfusion, glucose uptake rates had varying degrees of reduction in model group and intervention group, which reached the lowest value at 60 min after ischemia, then gradually increased. After 8 h of reperfusion, model group returned to the control level; at the same time, compared with the model group, after reperfusion, glucose concentration increased more severely in transfection group (between the two groups, there were significant differences, $P < 0.05$); and the recovery was slower; at 8 h after reperfusion, it was still not fully recovered to the control level; at 12 h after reperfusion, it was basically returned to the control level (**Table 4**).

## Discussion

We found that in the pre-CPB experiments [10, 11], after canine CPB myocardial ischemia and reperfusion, HIF-1α mRNA expression increased significantly in Model group and DMOG myocardial tissue. Early myocardial ischemia may be closely related to HIF-1α. In in-vitro experiments, we found that after ischemia and hypoxia, in hypoxia 60 min [11], HIF-1α mRNA expression level can be detected.

**Table 4.** Changes in myocardial GLU uptake rate after ischemia and reperfusion ($\bar{x}\pm s$)

| | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| Control group | 3.90±0.68 | 3.88±0.87 | 3.88±0.54 | 3.84±0.33 | 3.98±1.23 |
| Model group | 1.48±0.09$^{*}$ | 2.04±0.31$^{*,\Delta}$ | 2.88±0.20$^{*,\Delta}$ | 3.30±0.11$^{*,\Delta}$ | 3.86±0.30$^{\Delta}$ |
| Empty-vector group | 0.81±0.15$^{*,\#}$ | 1.24±0.02$^{*,\#,\Delta}$ | 1.99±0.23$^{*,\#,\Delta}$ | 2.72±0.15$^{*,\#,\Delta}$ | 3.31±0.37$^{*,\Delta}$ |
| Transfection group | 0.86±0.19$^{*,\#}$ | 1.34±0.02$^{*,\#,\Delta}$ | 2.20±0.23$^{*,\#,\Delta}$ | 2.82±0.25$^{*,\#,\Delta}$ | 3.51±0.57$^{*,\Delta}$ |

Note: Compared with control group, $^{*}P < 0.05$; Compared with model group, $\#P < 0.05$; Compared with T1 time point, $^{\Delta}P < 0.05$.

Compared with the control group, model group was significantly higher ($P < 0.05$). When it was 15 min at perfusion, HIF-1α mRNA expression level was further increased. When it came to 60 min, the expression level reached its peak. When it came to 180 minutes, its expression level was still higher than the control group ($P < 0.05$). When it came to 8 h, the expression level basically decreased to the control group level. In transfection group, it was expected to reduce the expression level of HIF-1α mRNA. We tried to reduce its expression level or keep it without expression in gene level. Currently the liposomal transfection efficiency, after we calculated, was about 73%. We cannot guarantee it 100% of silence. After transfection, there were still some myocardial which experienced ischemia and hypoxia with increasing expression level. The trend was similar with model group, and the expression level reached its peak 60 min after reperfusion. 8 h after reperfusion, its expression level restored to the control groups'. In the Western Blot test, we can see very little or basically no protein expression in the control group. After 60 min of hypoxia, the bands were obvious in the model group and transfection group. When the reperfusion increased to 60~180 min, it reached its peak. At T5 time when it continued to perfuse for 8 h, gray stripe significantly darken, but it still not recovered to the level of control group. The stripe was significantly gray in transfection group stripe. Compared with the model group, it was a little paler, which was also consistent with the RT-PCR results. Pisani studies suggested that Hypoxia-induced HIF-1α adjustment mainly occurred in post-transcriptional protein levels [12]. Our study showed that HIF-1α expression was more intense and with longer duration at the protein level.

Our further study found that low expression of IRI myocardial IR and GLUT-4 correlated with translocation abnormalities [13, 14]. Adding rosiglitazone in cardioplegia solution can improve CPB IRI myocardial GLUT4 mRNA expression, alleviate IRI myocardial IR and improve cardiac function [15]. In in vitro experiments, RT-PCR detection showed that in early ischemia reperfusion, expression of cardiomyocytes GLUT-4 was reduced. After HIF-1α silenced, this trend was more obvious and severe. We can understand this trend. In the control group at each time points the distribution of GLUT-4 cell protein fluorescence was uniform. We can see ischemia indexable situation after reperfusion and cell distribution after immunofluorescence from the picture and fluorescence intensity. GLUT-4 protein in the control group at each time point was mainly within the cell membrane. However, some of them distributed on the cell membrane. And the distribution is more uniform without significant difference. After hypoxia ischemia, experiments showed that after hypoxia, GLUT-4 gene expression was reduced. Protein transport barrier existed, which was consistent with in vivo experimental results [15]. After reperfusion, it had been restored. However, in a short time, the degree of recovery was slow. It returned to a normal state 8 h later. After HIF-1αsi-RNA transfection, the majority of HIF-1α was silent and did not express. GLUT-4 protein transport barrier, compared to ischemia-reperfusion group, was more obvious and the duration was longer. When the controller HIF-1α gene was silent, GLUT-4 protein transport barrier aggravated.

After ischemia, from morphological viewpoint of myocardial cells in model group, part of cardiac cells cracked to death and part of them shrank intensely. Stripes disappeared in some cells. In HIF-1αsi-RNA transfection group cardiomyocytes showed higher mortality ($P < 0.05$) after experiencing ischemia and hypoxia. The intensity of shrinkage was more intense, in addition to ischemia-reperfusion injury, HIF-1α expression and myocardial cell protection were reduced which may be the reasons leading to

death. From the viewpoint of GLUT-4 expression distribution, we can see that in the control group its distribution was more uniform, most of which were in intracellular. After experiencing ischemia in model group, we found that the distribution GLUT-4 on cell membrane reduced significantly and myocardial cell expression was stronger. In HIF-1αsi-RNA transfection group, this trend was more obvious and distribution of GLUT-4 on the cell membrane was less. So when it was in hypoxia, the expression of hypoxia controlling element HIF-1α was significantly increased. When its expression was silent, GLUT-4 expression in myocardial cell was significantly increased compared with the model group and its expression on the cell membrane decrease significantly compared with the model group.

The experimental results showed that when HIF-1α silenced, its expression was significantly reduced. After ischemia and reperfusion, the number of cardiomyocytes and the survival rate were decreased. GLUT-4 translocation barriers increased, and the utilization for Glu was decreased. The performance of insulin degradation was more and more severe. Insulin resistance was more obvious during metabolism, and the obstacle on the utilization for Glu was more obvious, indicating that HIF-1α played a central role in regulating this mechanism. HIF-1α may be one of the molecular mechanisms that triggered myocardial IR.

## Acknowledgements

This study was supported by National Natural Science Foundation of China (30960382). This project is sponsored by national natural science foundation (Project No.: 8156020262).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Gui-You Liang, Department of Thoracic and Cardiovascular Surgery, Affiliated Hospital of Zunyi Medical College, Zunyi 563003, P. R. China. Tel: +86-852-3146018; Fax: +86-852-3146018; E-mail: guiyou5150@163.com

## References

[1] Hausenloy DJ, Boston-Griffiths E, Yellon DM. Cardioprotection during cardiac surgery. Cardiovasc Res 2012; 94: 253-265

[2] Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med 2007; 357: 2408.

[3] Liu X, Huang Z, Zou X, Yang Y, Qiu Y, Wen Y. Possible mechanism of PNS protection against cisplatin-induced nephrotoxicity in rat models. Toxicol Mech Methods 2015; 19: 1-19

[4] Lin F, Pan LH, Ruan L, Qian W, Liang R, Ge WY, Huang B. Differential expression of HIF-1α, AQP-1, and VEGF under acute hypoxic conditions in the non-ventilated lung of a one-lung ventilation rat model. Life Sci 2015; 124: 50-5

[5] Flannigan KL, Agbor TA, Motta JP, Ferraz JG, Wang R, Buret AG, Wallace JL. Proresolution effects of hydrogen sulfide during colitis are mediated through hypoxia-inducible factor-1α. FASEB J 2015; 29: 1591-602

[6] Bajgelman MC, Dos Santos L, Silva GJ, Nakamuta J, Sirvente RA, Chaves M, Krieger JE, Strauss BE. Preservation of cardiac function in left ventricle cardiac hypertrophy using an AAV vector which provides VEGF-A expression in response to p53. Virology 2014; 476: 106-114.

[7] Xie HC, He JP, Zhu JF, Li JG. Expression of HIF-1alpha and VEGF in skeletal muscle of plateau animals in response to hypoxic stress. Physiol Res 2014; 63: 801-5.

[8] Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 2012; 148: 399-408

[9] Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, Thistlethwaite PA. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. N Engl J Med 2000; 342: 626-633.

[10] Liang GY, WU HS, Li J, Ca QY, Gao ZY. Role of insulin receptors in myocard ischaemia reperfusion injury during cardiopulmonary bypass. Acta Cardiol 2011; 66: 323-331.

[11] Pisani DF, Dechesne CA. Skeletal muscle HIF-1alpha expression is dependent on muscle fiber type. J Gen Physiol 2005; 126: 173-178.

[12] Vincent MA, Clerk LH, lindner JR, Klibanov AL, Clark MG, Rattigan S, Barrett EJ. Microvascular recruitment is an early insulin effect that reulates skeletal muscle lucose uptake in vivo. Diabetes 2004; 53: 1418-1423.

[13] Russell RR, Li J, Coven DL, Pypaert M, Zechner C, Palmeri M, Giordano FJ, Mu J, Birnbaum MJ, Young LH. AMP-activated protein kinase mediates ischemic lucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. J Clin Invest 2004; 114: 495-503.

[14] Liu B, Liang GY, Xu G, Liu D, Cai Q, Gao Z. Intervention of rosiglitazone on myocardium Glut-4 mRNA during ischemia-reperfusion injury in cardio-pulmonary bypass in dogs. Mol Cell Biochem 2013; 73: 279-284.

[15] Liang GY, Cai QY, Niu YM, Zheng H, Gao ZY, Liu DX, Xu G. Cardiac glucose uptake and suppresses expression/translocation of myocardium glucose transport-4 in dogs undergoing ischemia-reperfusion. Exp Biol Med 2008; 233: 1142-1148.