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*Original Article*

# Lentiviral vector *PLV-PI3KCG* gene transfer inhibits hypoxic cardiomyocytes apoptosis

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**Abstract:** The PI3K/Akt signal pathway was suggested to be associated with apoptosis. However, it was still unclear whether activated PI3K/Akt signaling pathway could inhibit hypoxic cardiomyocytes apoptosis. In this research, the recombinant *PI3KCG* lentiviral vector plasmid (*PLV-PI3KCG*) was constructed and transfected into neonatal rat hypoxia/reoxygenation (H/R) injury cardiomyocytes models which were randomly divided into five groups as the normal control group, H/R group, HR empty plasmid group (HRE group), HR *PLV-PI3KCG* transfection preconditioning group (HRP group), and HR *PLV-PI3KCG* transfection + LY294002 group (HRPL group). Compared with the H/R, HRE and HRPL groups, the cardiomyocytes beat frequency and survival rate in the HRP group were significantly increased ($P<0.05$) and the released LDH were significantly decreased ($P<0.05$). The Bcl-2/Bax ratio was significantly lower in H/R, HRE and HRPL groups than that in HRP group ($P<0.05$). Activated PI3K/Akt signaling pathway could play a protection role in the cardiomyocytes H/R injury process which could be inhibited by LY294002.

**Keywords:** Lentiviral vector, *PI3KCG*, virus packaging, cardiomyocytes, hypoxia/reoxygenation

## Introduction

Cardiomyocyte apoptosis participates in various cardiac diseases including acute myocardial infarction, hypoxia/reperfusion injury, cardiac allograft, and heart failure. The studies on the cardiomyocyte apoptosis mechanism and inhibition approach have become the research focus [1]. Researches have shown that as one of the important cellular signal pathways, phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) signal pathway plays a key role in the regulating cell cycle course, differentiation and survival [2, 3]. However, the concrete mechanism of PI3K/Akt signal pathway activation preventing the cardiomyocytes hypoxia/reoxygenation (H/R) injury was still unclear. In the current research, the lentiviral vector containing human *phosphatidylinositol 3-kinase, catalytic subunit gamma* (*PI3KCG*) gene has been constructed and identified by using lentiviral package technology. Moreover, the recombinant *PI3KCG* lentiviral vector plasmid *pLenti6/V5-PI3KCG* (*PLV-PI3KCG*) was transfected into neonatal rat cardiomyocytes and intervened by H/R procedure to analyze the *PI3KCG* gene function. It will lay the foundation for the following in vivo study on the protection mechanism of *PI3KCG* gene in AMI progress.

## Material and methods

### *Construction of recombinant PLV-PI3KCG lentiviral vector*

The *PLV-PI3KCG* was constructed by gateway LR homologous recombination method in vitro. The LR Clonase™ II enzyme was unfrozen and mixed with vortex on ice. The *pEntry-PI3KCG* plasmid (Inventrogen Inc, 150 ng, 1 μL), pLenti6/V5-DEST (Invitrogen Inc, 150 ng, 1 μL), TE buffer (30 mM Tris·Cl, 1 mM EDTA, pH 8.0, 8 μL) were added into the 1.5 mL centrifuge tube successively and mixed at room temperature which was joined by 2 μL LR Clonase™ II enzyme mixture soon afterwards, mixed together again and incubated for 1 hour at room temperature. 1 μL Proteinase K solution was then added into the above mixture and the reaction was terminated at 37°C for 10 minutes. The above termination reaction solution was taken out for 2 μL which was put into 100 μL competence XL-BLUE

**Table 1.** The sequence table of the PCR primers

| Primers name | Primers sequence 5' to 3' |
|---|---|
| PI3KCG-F1 | CATCCCCATCGAGTTCGTG |
| PI3KCG-R1 | CAGTTG TTGGCAATCTTCTTCC |
| PI3KCG-F2 | AACCTATTTCATATTGACTTCGGG |
| PI3KCG-R2 | AATTAAACTGCACAG TCCATCCTT |
| CMV-F | CGCAAATGGGCGGTAGGCGTG |
| V5 reverse | ACCGAGGAGAGGGTTAGGGAT |

Escherichia coli together and absorbed for 30 minutes on ice. Then 2 μL termination reaction solution and Escherichia coli mixture was swashed for 50 seconds at 42°C which were rapidly cooled for 2 minutes on ice, added by 1 mL LB culture medium, oscillated for 1 hour at 37°C by 180 rpm and then centrifuged for 3 minutes by 6000 rpm. The supernatant were abandoned and the remaining mixture was mixed with 200 mL LB culture medium. The 100 mL solution were taken out of the above mixture, coated onto the LB culture plating with 100 μg/mL ampicillin and cultured overnight at 37°C. Ten clones were picked out on the second day, inoculated into 3 mL LB culture medium with 100 μg/mL ampicillin, shaken and cultured overnight at 37°C by 250 rpm.

*Positive clones identification by colony PCR and bi-directional sequencing verification of positive clones*

The recombinant *PLV-PI3KCG* lentiviral vector genomic DNA was extracted. The PCR reaction was performed to identify the *PLV-PI3KCG* lentiviral vector by using *PI3KCG* gene primers. The upstream and downstream primers were PI3KCG-F1, PI3KCG-R1, PI3KCG-F2 and PI3KCG-R2. The reaction system were 20 μL including 10× Buffer 2 μL, $Mg^{2+}$ (25 mmol/L) 2 μL, dNTPs 0.4 μL, upstream and downstream primers (5 μmol/L) 1 μL respectively, Taq enzyme (5 U/μL) 0.1 μL, $ddH_2O$ 12.5 μL, and template 1 μL. The PCR amplification procedure is pre-denaturation for 3 minutes at 94°C, denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C, extension for 30 seconds at 72°C for 35 cycles and extension for 7 minutes at 72°C at last. The positive clones were bi-directionally sequenced and verified by using CMV-F and V5 reverse primers (**Table 1**).

*Recombinant PLV-PI3KCG lentiviral vector package in HEK 293T cells and identification*

The 10 cm culture dish was coated by gelatin (Sigma Inc, USA) for 24 hours before transfection. The well-grown HEK 293T cells were inoculated into the culture dish at 37°C and 5% $CO_2$. When the 293T cells were grown to about 85% confluence, the recombinant *PLV-PI3KCG* lentiviral vector or empty lentiviral vector was transfected into the 293T cells by using Lipofectamin™ 2000 reagent (Invitrogen Inc, USA) and the null plasmid with GFP was also transfected at the same time as the control to observe the transfection effect. The opti-MEM (without serum and antibiotics) were added into two Eppendorf tubes, of which the DNA (conjugative plasmid: recombinant *PLV-PI3KCG* plasmid, package diolame pLP1, pLP2 and VSVG, Addgene Inc) were added into one Eppendorf tube and Lipofectamin™ 2000 was added into another Eppendorf tube. The solutions of the two Eppendorf tubes were mixed for 20 minutes at room temperature, added into the 10 cm culture dish and shaken up. The viral solution was collected after incubation for 48 hours and filtrated by 0.45 μm filter membrane. The 5 μL viral solution was picked out and used to perform PCR amplification and sequence to identify the target gene expression. The PCR amplification primers and condition were the same to that in the above mentioned text. The remained viral solution was stored for standby application at -80°C.

*The cardiomyocytes preparation and hypoxia model establishment in vitro [4]*

The present research was approved by the ethnic committee of the First Affiliated Hospital of Nanjing Medical University. Cardiomyocytes were prepared from 1- to 3-day-old neonatal Sprague-Dawley rats (150-180 g, Nanjing Qinglongshan Multiplying Farm, China). The chest was opened after the rat was washed by alcohol and the heart was taken out and put into phosphate buffer solution (PBS) at 4°C. The blood stasis was removed by washing repeatedly and the atrial tissue was cut off. The remained ventricular tissue was digested repeatedly by adding 0.1% trypsin solution and the cardiomyocytes were isolated by differential attachment method. The cardiomyocytes were inoculated into 24-well plates at a density

of $1\times10^5$ cells/mL in Dulbecco modified Eagle medium (DMEM) supplemented with 15% fetal bovine serum. After 72 hours incubation at 37°C and 5% $CO_2$, cardiomyocytes were grown to about 80% confluence, followed by random allocation experiments. Cardiomyocytes were then transfected at 50 MOI with empty lentiviral vector, *PLV-PI3KCG*, or *PLV-PI3KCG* + LY-294002 (10 mM) at $10^5$ pfu/mL by adding 10 mg/L polybrene transfection reagent (Merk Inc, German). After 12 hours transfection, the viral transfection solution was replaced by DMEM with 10% calf serum. The isolated cardiomyocytes were subsequently random divided into five groups after being cultured for 3 days: the normal control group, H/R group, HRE group, HRP group, and HRPL group. The same experiments were repeated for five times.

The H/R injury model of neonatal rat myocardial cells was established. After the cardiomyocytes were co-cultured with recombinant *PLV-PI3KCG* or empty plasmid for 72-96 hours, except the control group, all of the other four groups were treated with hypoxia for 180 minutes by using hypoxia bag (Becton Dickinsonand Inc, USA) (95% $N_2$ and 5% $CO_2$) and they were then dealt with reoxygenation for 120 minutes (70% $N_2$, 25% $O_2$, and 5% $CO_2$) to simulate the H/R process.

*Identification of PI3KCG gene in the transfected cardiomyocytes*

The total RNA was extracted from the transfected cardiomyocytes. The RT-PCR reaction was performed to identify the *PLV-PI3KCG* lentiviral vector by using CMV-F and V5 reverse primers which were the same to the above mentioned primers (**Table 1**). The RT-PCR products were sequenced subsequently.

*Analysis of LDH in cardiomyocytes*

The LDH detection kit (Nanjing Jiancheng Bioengineering Institute) was used to analyze LDH level. Measurements of LDH from cell supernatants were analyzed by full-automatic biochemical analyzer (Beckman, USA).

*Cardiomyocytes survival rate assay by cell count kit-8 method (CCK-8, WST-8) [5]*

The cardiomyocytes were inoculated into 96-well plates with 100 μL suspension per cell. Additionally, 100 μL DMEM per well was acted as the blank control group. The five group experiment was performed as the above mentioned. After the H/R procedure, CCK-8 was added by 10 μL/well and incubated with cardiomyocytes for another 0.5 hour at 37°C. The 450 nm wave length of microplate reader was selected and absorbance value A was measured. The cardiomyocytes survival rate was calculated by the following formula: viable cell percent (%)=(value A of experiment group/value A of control group) ×100%.

*Cardiomyocyte apoptosis assay by western blot*

The cardiomyocytes were inoculated into 6-well plates with 2 ml suspension per cell. After the H/R procedure, DMEM was discarded, and the cardiomyocytes were twice rinsed and digested by trypsin. The cardiomyocytes were collected by 1200 g centrifuging for 3 minutes and added by 0.1 mL RIPA Lysis buffer, 1 μL protease inhibitor, and 1 μL PMSF. The cardiomyocytes and lysate were transferred into 1.5 mL Eppendorf tubes, placed on ice for 5 minutes and centrifuged by 13000 g for 5 minutes at 4°C. The supernatant was transferred into new Eppendorf tubes. The protein concentration of the supernatant was measured by BCA protein measure kit and BSA standard substance. Then the protein (50 μg) was separated by 10% SDS-PAGE gel, transferred to PVDF membrane and sealed by 5% skim milk powder for 2 hours. The 1:1000 diluted rabbit anti-rat antibodies as β-Actin, PI3KCG, Bax and Bcl-2 (Cell Signaling Technology Inc, USA) were incubated with the transferred PVDF membrane overnight at 4°C. β-Actin antibody acted as internal reference antibody. After PVDF membrane was washed by Tris-Buffered-Saline with Tween (TBST), 1:2000 diluted goat anti rabbit IgG-HRP (Santa Cruz Biotechnology Inc, USA) were added and incubated with the transferred PVDF for 40 minutes at 37°C. After the PVDF membrane was washed by TBST for three times, it was exposed by ECL for 1 minute to perform the band density analysis. Image Lab software was used to analyze the band and perform the protein quantification.

*Statistical analysis*

The SPSS software system (SPSS for Windows, version 10.0) was used to perform the statistical analysis. Measurement data were expressed

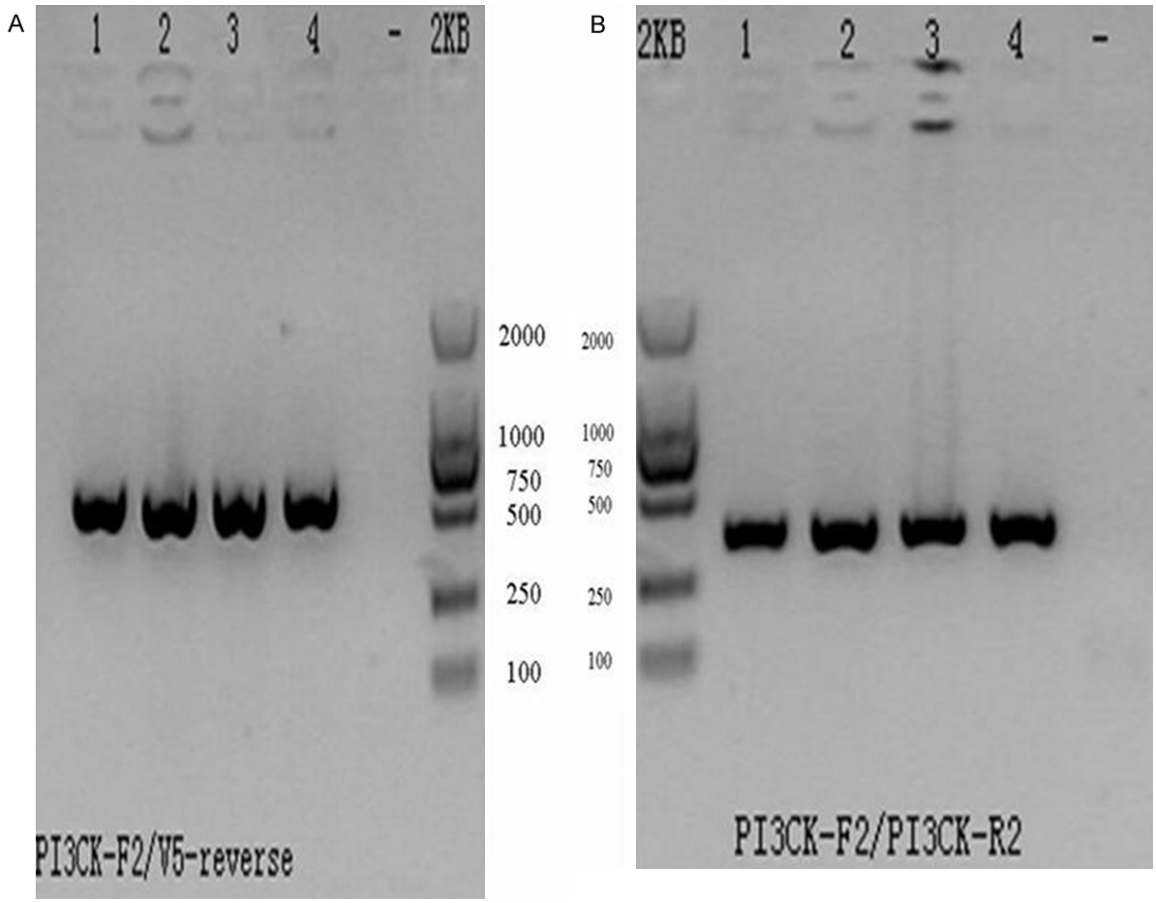


**Figure 1.** The recombinant *PLV-PI3KCG* plasmid was identified by PCR reaction. A. The PCR amplification agarose gel electrophoretogram of the positive clone of the transfected HEK293 cells by using *PI3KCG-F2/V5* primers. Lane 1-4: The PCR amplification products by using *PI3KCG-F2/V5* primers (540 bp); Lane-: The blank control by using $H_2O$. B. The PCR amplification agarose gel electrophoretogram of the positive clone of the transfected HEK293 cells by using *PI3KCG-F2/R2* primers. Lane 1-4: The PCR amplification products by using *PI3KCG-F2/R2* primers (382 bp); Lane-: The blank control by using $H_2O$.

as mean ± standard deviation (mean ± SD). One-way analysis of variance (ANOVA) followed by Dunnett's test. A statistically significant difference was defined as $P<0.05$.

## Results

### *The PLV-PI3KCG plasmid construction and polymerase chain reaction (PCR) identification*

The objective bands were distinctly observed in the 540 bp and 382 bp after the agarose gel electrophoresis for PCR products of the transfected recombinant *PLV-PI3KCG* lentiviral plasmid positive clones. The band size was in agreement with the expected results. The sequencing results were completely in agreement with the given human *PI3KCG* gene sequence which suggested the recombinant *PLV-PI3KCG* lentiviral plasmid was successfully constructed (**Figure 1A**, **1B**). **Figure 2A** depicted the original pLenti6/V5-DEST vector map and **Figure 2B** displayed the recombinant *PLV-PI3KCG* lentiviral plasmid map.

### *Recombinant PLV-PI3KCG plasmid package and identification*

The lipofection transfection method was used to co-transfect the conjugative plasmid *PLV-PI3KCG* or empty plasmid and packaging diolame plasmid into human embryonic kidney (HEK) 293T cells. The GFP protein expression could be observed in the empty lentiviral plasmid group after transfection for 24 hours by fluorescence microscopy. The GFP protein fluorescence intensity was further strengthened after transfection for 48 hours. The positive

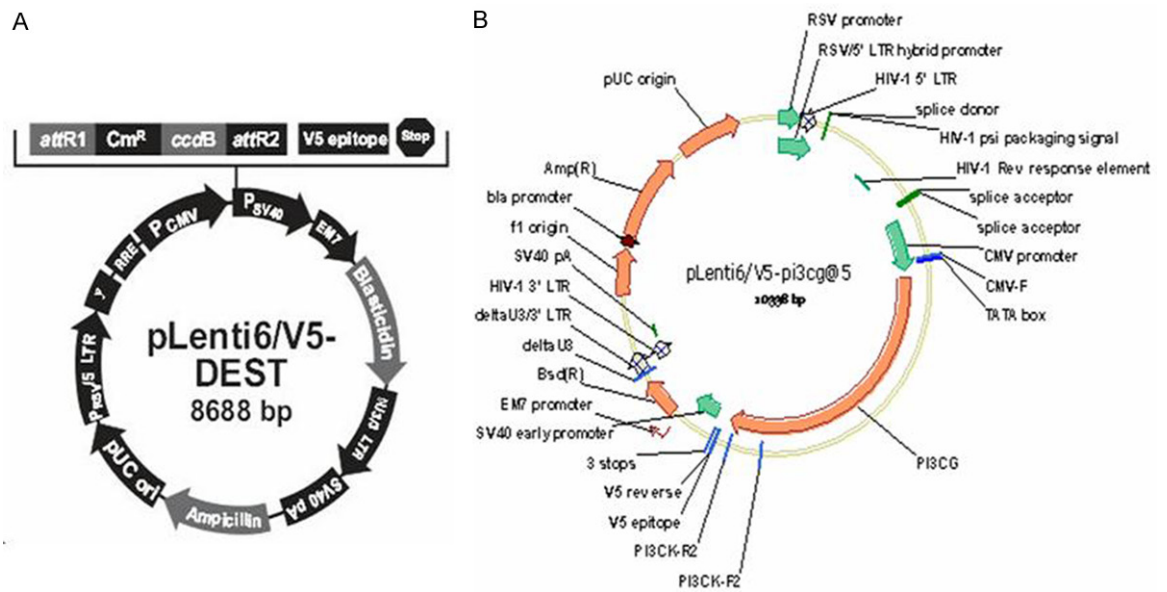


**Figure 2.** The vector map used in the current research. A. Map of pLenti6/V5-DEST; B. Map of recombinant *PLV-PI3KCG* vector.

package ratio of GFP protein expression was up to 100% after transfection for 72 hours which implied that the *PLV-PI3KCG* was successfully packaged. It was verified that the *PI3KCG* gene was contained in the recombinant lentiviral genome by PCR and sequencing which suggested that the recombinant *PLV-PI3KCG* or empty plasmid was successfully transfected into HEK 293T cells. The virus titer of the recombinant *PLV-PI3KCG* or empty plasmid was up to $10^6$ IU/mL after the transfected HEK 293T cells were multi-round amplified.

*Identification results of PI3KCG gene in the transfected cardiomyocytes*

The transfected cardiomyocytes total RNA was sucessfully extracted and the reverse transcription PCR products were also observed in the 540 bp and 382 bp by agarose gel electrophoresis. The bi-directional sequencing results were completely in accordance with the *PI3KCG* gene which suggested the *PI3KCG* gene was successfully transfected into cardiomyocytes (**Figure 3**).

*The PI3KCG gene attenuated cardiomyocytes viability loss in the H/R procedure*

The cardiomyocyte did not adhere originally and presented as roundness which soon adhered and gradually presented as fusiformis and polygon. The cardiomyocyte size was uniform and grew up quickly. The single cardiomyocyte wriggled by itself and when many cardiomyocytes grew up to flakiness and beaten rhythmically like an island. After the H/R intervention, the cardiomyocytes survival rate and rhythm of the heart were significantly reduced than the control group ($P<0.05$). However, the cardiomyocytes survival rate and beat frequency in the *PLV-PI3KCG* transfection preconditioning group (HRP group) were significantly increased than that in the H/R, HR empty plasmid group (HRE) and HR *PLV-PI3KCG* transfection + LY294002 (HRPL) groups ($P<0.05$) (**Figure 4**).

*The PI3KCG gene attenuated lactate dehydrogenase (LDH) release amount*

The LDH release amount in the medium was measured as the cardiomyocytes apoptosis index. As shown in **Figure 4**, the LDH level of the cardiomyocytes medium supernatant was significantly higher in the H/R group than that in the control group ($P<0.05$). However, the LDH level was significantly lower in the HRP group than that in the H/R group ($P<0.05$). No significant difference was detected in LDH level among HRE, H/R or HRPL groups ($P>0.05$).

*The intracellular mechanisms of PI3KCG gene protecting cardiomyocytes against apoptosis in the H/R injury*

The western blot results of the transfected cardiomyocytes with recombinant *PLV-PI3KCG* plasmid in the H/R procedure were shown in

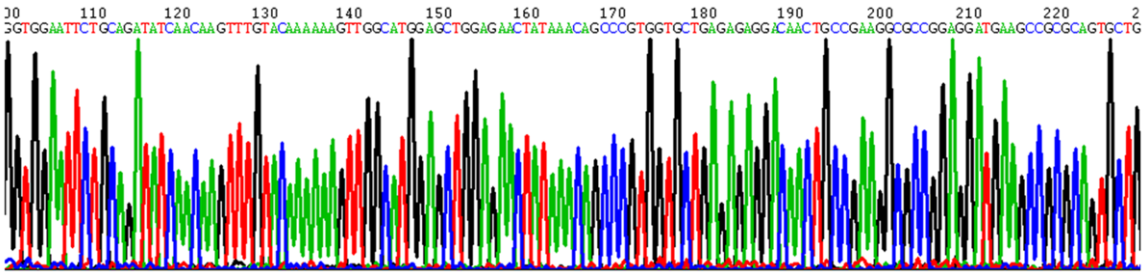

**Figure 3.** The RT-PCR products sequence identification report of *PI3KCG* gene in transfected cardiomyocytes.

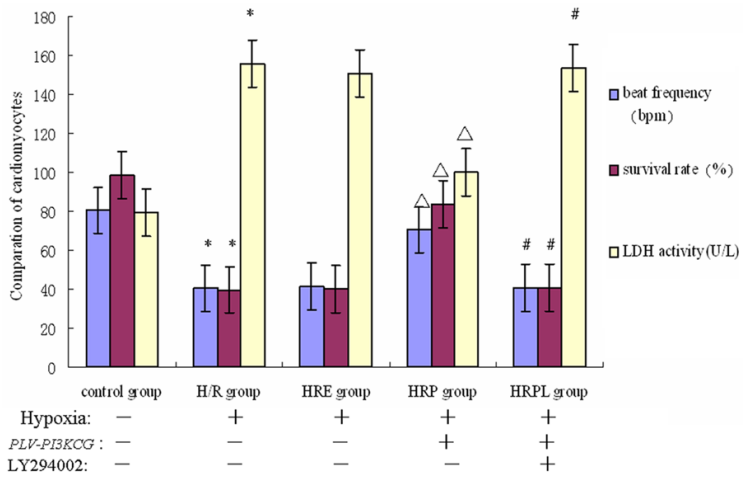


**Figure 4.** Comparation results of cardiomyocytes beat frequency, survival rate and supernatant LDH level between the five groups (n=5). *P<0.05 vs. control group; ΔP<0.05 vs. H/R group; #P<0.05 vs. HRP group.

**Figure 5.** The apoptosis-promoting protein (Bax) was highly expressed in H/R, HRE and HRPL groups which were significantly decreased in HRP group. By contrast, the apoptosis-inhibiting proteins (Bcl-2) were lowly expressed in H/R, HRE and HRPL groups which were significantly increased in HRP group. The PI3KCG protein expression was also decreased in H/R and HRE groups which were significantly increased in HRP and HRPL groups. No significant difference was detected in the β-Actin protein expression among the five groups (**Figure 5A**).

**Figure 5B** has shown the PI3KCG/β-Actin ratio was decreased both in the H/R and HRE groups (0.060±0.004, 0.055±0.005, respectively, P<0.05, compared to control: 0.112±0.003) and this decrease was largely inhibited by recombinant *PLV-PI3KCG* plasmid administration in HRP and HRPL groups (0.090±0.002, 0.088±0.003, respectively, P<0.05, compared to H/R group). Compared with H/R group, no significant difference was detected in the HRE group (0.055±0.004, P>0.05, compared to H/R group). There was also no significant difference in PI3KCG/β-Actin ratio between HRP and HRPL groups (P>0.05).

**Figure 5C** depicted that the Bcl-2/Bax ratio was decreased in the HR group (0.20±0.02, P<0.05, compared to control: 0.47±0.03) and this decrease was greatly blocked by administration of recombinant *PLV-PI3KCG* plasmid in HRP group (0.36±0.04, P<0.05, compared to H/R group). In addition, the recombinant *PLV-PI3KCG* plasmid-mediated Bcl-2 protein increase and Bax protein decrease were abrogated by the PI3K inhibitor LY294002 treatment in HRPL group (0.18±0.02, P<0.05, compared to HRP group). Compared with H/R and HRPL group, no significant difference was detected in the HRE group (0.16±0.03, P<0.05, compared to HRP group).

## Discussion

In the current research, the recombinant *PLV-PI3KCG* plasmid was successfully constructed and packaged. The transfected *PI3KCG* gene function was also confirmed to have the protection effect on the hypoxia cardiomyocytes. The human *PI3KCG* gene spans 3.3 kb which is located in 7q22.3. Given that the *PI3KCG* gene is relatively large, the green fluorescent protein (GFP) gene of the pLenti6/V5-DEST lentiviral vector was removed by enzyme digestion to avoid influencing the *PI3KCG* gene function in the recombinant *PLV-PI3KCG* plasmid. Hence, in the host HEK 293T cells transfected with *PLV-PI3KCG* plasmid, the GFP protein expres-

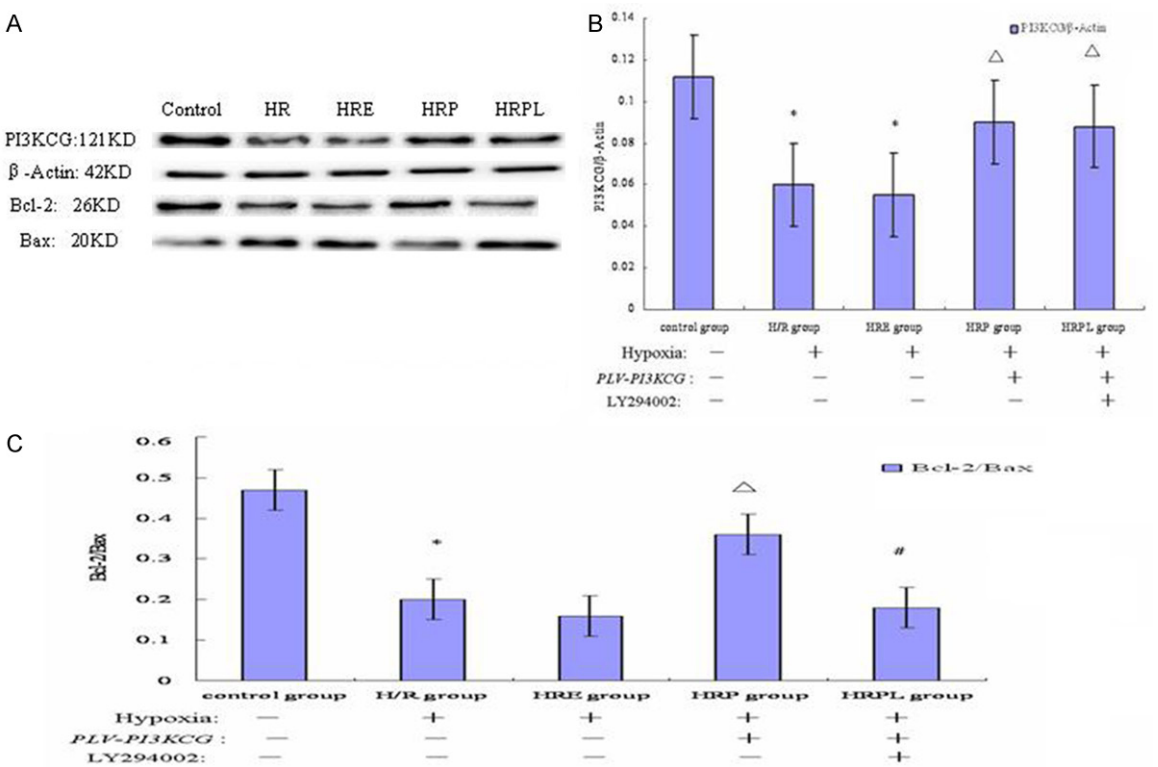


**Figure 5.** The intracellular mechanisms of *PI3KCG* gene protecting cardiomyocytes against apoptosis in the H/R injury. A. The western blot results of the transfected cardiomyocytes with recombinant *PLV-PI3KCG* plasmid in the H/R procedure. The effect of recombinant *PLV-PI3KCG* plasmid on the protein levels of PI3KCG, Bcl-2, and Bax. The recombinant *PLV-PI3KCG* plasmid mediated PI3KCG, Bcl-2 protein increase, and Bax protein decrease. The Bcl-2 protein increase and Bax protein decrease were abrogated by the PI3K inhibitor LY294002 treatment. Data are presented as the mean ± SD (n=5). The β-Actin was analyzed as loading control. B. The effect of recombinant *PLV-PI3KCG* plasmid on the PI3KCG/β-Actin protein ratio in hypoxia-induced cardiomyocytes apoptosis. The recombinant *PLV-PI3KCG* plasmid mediated PI3KCG protein increase. Data are presented as the mean ± SD. (n=5). *P<0.05 vs. control group; ΔP<0.05 vs. H/R group. C. The effect of recombinant *PLV-PI3KCG* plasmid on the Bcl-2/Bax protein ratio in hypoxia-induced cardiomyocyte apoptosis. The recombinant *PLV-PI3KCG* plasmid-mediated Bcl-2 protein increase and Bax protein decrease were abrogated by the PI3K inhibitor LY294002 treatment. Data are presented as the mean ± S.D. (n=5). *P<0.05 vs. control group; ΔP<0.05 vs. H/R group; #P<0.05 vs. HRP group.

sion could not be observed which could only be observed in the HEK 293T cells transfected with empty plasmid to see the transfection efficiency. The following PCR and sequence methods were used to determine whether the transfection process was successfully performed or not. In the *PLV-PI3KCG* plasmid recombination process, the traditional repeat digestion and link technologies were not adopted and the gateway homologous recombination method was used. Thus, the *PLV-PI3KCG* plasmid was constructed simply and efficiently [6].

The current research is the first study to explore the protection effect of *PI3KCG* gene on hypoxia cardiomyocytes by using lentiviral vector. As well known, there are many gene transfection methods as physical, chemical, retrovirus vector, adenovirus vector methods and so on. The transfection efficiency was low by using physical or chemical method. The retrovirus vector has the bad capacity to accommodate the exogenous gene and can only infect the division stage cells. Additionally, it is difficult for the adenovirus vector to realize the stable expression of the exogenous gene. The lentiviral vector, constructed on the basis of human immunodeficiency virus-1, has the large capacity to accommodate the exogenous gene, can realize the stable expression of the transferred gene and infect the non-division stage cells. The lentivirus titer demanding for transfection is high up to $1\times10^7$-$1\times10^8$ IU/mL with little host immunoreaction. The aforementioned merits of the lentiviral vector overcome the deficiency of the retrovirus and adenovirus vectors.

The treatment of acute myocardial infarction (AMI) with thrombolysis and percutaneous transluminal angioplasty methods always lead to the ischemia reperfusion injury, especially cell apoptosis and necrosis which caused the patients condition worsened. Hence, inhibition of cardiomyocytes apoptosis becomes the main way for the treatment of AMI and congestive heart failure sequela [7]. PI3K/Akt is the important celluar signal transduction pathway. The activated PI3K/Akt signal pathway could further active or inhibit the downstream target protein by phosphorylation, thus play the role of regulating cell proliferation, differentiation, glucose metabolism and migration [8-20]. In 2007, Arab et al found that in the reversible myocardial ischemia-reperfusion during cardiac surgery, the ischemia preconditioning could cause the Akt phosphorylation level increase. The PI3K inhibitors such as Wortmannin and LY-294002 could enlarge the myocardial infarction zone, inhibit the ischemic cardiac function improvement and reduce the phosphorylation level [21]. Murphy et al reported that lack of PI3K could not help the cardiac function recover during the ischemia reperfusion procedure, increased the dead cells number, and weakened the adenosine protection effect [22]. Matsui et al found that hypoxia induced cell apoptosis could be reduced by transfecting the *PI3K* or *Akt* gene into cardiomyocytes using adenovirus vector [23]. In 2010, Li et al reported that Asperosaponin VI had a protective effect against hypoxia-induced cardiomyocytes apoptosis probably by activating the PI3K/Akt and CREB pathways [24].

The current research results have shown that the cardiomyocytes survival rate in the H/R group was significantly reduced and LDH release quantity was significantly increased compared with control group ($P<0.05$) which suggested that the cardiomyocytes H/R injury model was successfully constructed. After the recombinant *PLV-PI3KCG* plasmid was transfected into cardiomyocytes, PI3KCG protein overexpression was detected in HRP and HRPL groups which suggested that the *PLV-PI3KCG* plasmid was successfully constructed, transfected and expressed. In the following function test, it was observed that the cardiomyocytes beat frequency, survival rate, and Bcl-2 protein expression in the HRP group was significantly increased than that in the H/R, HRE and HRPL groups. The LDH release amount and Bax protein expression in the HRP group were significantly decreased than that in the H/R, HRE and HRPL groups. It was verified that in the present study that *PI3KCG* gene has the significant protection effect on the cardiomyocytes hypoxia reoxygenation injury by activating the PI3K/Akt signal pathway. In addition, the protection effect was lost after the PI3KCG inhibitor LY294002 was added.

There are still some limitations in the present research. The current study was just an experiment in vitro. The in vivo research on the *PI3KCG* gene protection effect on the cardiomyocytes hypoxia reoxygenation injury still needed to be carried out in the near future. Furthermore, the protection effect was possibly mediated by other cytokines as transforming growth factor-beta 1 which needed to be verified by more studies.

Taken together, *PLV-PI3KCG* plasmid was expected to become an available vector to investigate PI3K/Akt pathway in the cardiomyocytes H/R injury process. *PI3KCG* gene was markedly associated with the protection effect on the cardiomyocytes H/R injury. The current conclusion might help us to formulate novel ischemia coronary disease therapy strategies. Given the limitations, more studies in vivo are needed to clarify the significance of the conclusion.

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## Disclosure of conflict of interest

None.

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