

Knockdown of C3G Mitigates Post-Infarct Remodeling via Regulation of ERK1/2, Bcl-2 and Bax in Rat Myocardial Cells

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Abstract

Background: Cardiomyocyte-specific knockout of pro-survival integrin $\beta 1$ subunit and its downstream components have been demonstrated to aggravate remodeling after myocardial infarction (MI). However, as a component of integrin pathway, it is unclear whether knockdown of pro-survival C3G (rap guanine nucleotide exchange factor 1) in cardiac myocytes and fibroblasts could have effect on myocardial remodeling.

Methods and results: A rat model of MI was established by ligation of left anterior descending coronary artery. Infarcted myocardium and its border zones in Sprague-Dawley rats were transiently infected with C3G knockout lentivirus via local injection to knockdown C3G in the myocardium. Twelve weeks after injection with the lentiviruses, cardiomyocytic apoptosis and collagen in surviving myocardium, and left ventricle (LV) end-diastolic diameter were decreased, whereas LV weight / body weight ratio and LV ejection fraction were increased in MI group via down-regulation of pro-survival C3G, phosphorylated (p) ERK1/2 (phosphorylated extracellular regulated kinase 1/2) and Bcl-2 (B-cell lymphoma-2), and up-regulation of pro-apoptotic Bax in the surviving myocardium. On the other hand, treatment with the lentiviruses was found to delete C3G and diminish cell proliferation *in vitro* cardiac myocytic and fibroblastic cell lines respectively via down-regulation of p-ERK1/2 and Bcl-2, and up-regulation of Bax.

Conclusions: Knockdown of pro-survival C3G in myocardium may mitigate surviving myocardial remodeling after MI, possibly through regulation of p-ERK1/2, Bcl-2 and Bax *in vivo* cardiac myocytes and fibroblasts.

Introduction

Myocardial remodeling, ischemic cardiomyopathy and left ventricular dysfunction are related to the increased risk of adverse cardiovascular events and mortality after myocardial infarction (MI) [1]. Pathological changes in the heart are determined by the improperly over-activated pro-survival genes *in vivo* cardiac myocytes and fibroblasts in the surviving myocardium around the infarcted lesion [2]. Cardiomyocyte-specific knockout of pro-survival integrin $\beta 1$ subunit and its downstream molecules such as focal adhesion kinase (FAK) have been found to exacerbate myocardial remodeling after MI via their aggravation of apoptosis in the cardiomyocytes [3–5]. Pro-survival adaptor protein CrkL (v-crak avian sarcoma virus CT-10 oncogene homolog (Crk)-like) can bind to C3G (rap guanine nucleotide exchange factor 1), which acts as a guanine nucleotide exchanger in integrin pathway [3]. Our previous study has shown that knockdown of CrkL can worsen cell apoptosis, which was induced by hypoxia / reoxygenation (H/R) *in vitro* cardiomyocytic cell line [6]. The effects of CrkL are inferred to be mediated by its downstream pro-survival molecules such as C3G exchange factor. In addition, C3G protein has been shown to express *in vitro* cardiomyocytic cell line, and its elevated expression was confirmed in the surviving myocardium after MI [7, 8]; and knockdown of pro-survival C3G can aggravate H/R-induced apoptosis and pathological processes *in vitro* cardiomyocytic cell line [7]. However, it is still unclear whether knockdown of pro-survival C3G in myocardium may have effect on the process of surviving

myocardial remodeling after MI via regulation of p-ERK1/2 (phosphorylated extracellular regulated kinase 1/2), Bcl-2 (B-cell lymphoma-2) and Bax in cardiac myocytes and fibroblasts.

Materials And Methods

Construction of C3G knockout recombinant lentiviruses

The candidate sequences knocking specifically all 13 variant mRNA transcripts of rat *C3G* gene were designed (<http://www.e-crisp.org/E-CRISP/>) [7] and cloned respectively into pLenti-Cas-Guide lentiviral vector (Origene Co., Ltd, Rockville MD, USA). RT-PCR (reverse transcription polymerase chain reaction) and protein immunoblot screening experiments revealed that the recombinant vector containing the targeting sequence was the most effective one to knockout *C3G* gene in the rat-derived H9C2 cardiac myoblastic cell line (Cat. No. CRL-1446, purchased from American Type Culture Collection, Manassas, VA, USA), and was used in subsequent experiments to knockout rat endogenous *C3G* mRNA (Table 1). The non-target (NT) was also cloned into the pLenti-Cas-Guide lentiviral vector, and formed a NT recombinant lentiviral vector, which was used for negative control (Table 1).

Table 1

The nucleotide sequence targeted by recombinant lentiviruses and primers for reverse transcription polymerase chain reaction

Lentiviruses or Gene primers	Sequences	Product Size (bp)	Tm (°C)
<i>C3G</i> KO	Sense: 5'-AAGAGAACACCATCCAAGAA-3'		48
NT	Sense: 5'-CTGGACCAGGCAGCAGCGTC- AGAAGACTTTTTTGGAA-3'		59
<i>C3G</i> primer	Sense: 5'-CAGGATGGACAGCAGACAGA-3' Anti-sense: 5'-CTGCGGTGTCTGGTAGAACA-3'	384	58
<i>GAPDH</i> primer	Sense: 5'-AGAACATCATCCCTGCATCC-3' Anti-sense: 5'-GGATGGAATTGTGAGGGAGA-3'	519	57
Note. <i>C3G</i> KO: <i>C3G</i> knockout recombinant lentivirus; NT: non-target knockout recombinant lentivirus; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.			

The *C3G* knockout (KO) and NT recombinant vectors were packaged into CRISPR (clustered regularly interspaced short palindromic repeats) / Cas9 recombinant lentiviruses ($\times 10^7$ TU/ml) respectively in 293T cells through using Lenti-vpak Lentiviral Packaging Kit, and selected respectively. DNA sequencing experiment revealed that the sequence knocking out *C3G* gene was successfully inserted into the recombinant lentivirus.

Cell culture in vitro and treatment with C3G knockout lentivirus and hypoxia

Primary cardiac fibroblastic cell line (Cat. No. 160927, Cell Applications Inc, San Diego, CA, USA) and H9C2 cardiac myoblastic cell line were cultured as described previously [7, 9]. An equal number of the cells (1×10^5) were infected with *C3G* KO and NT recombinant lentiviruses respectively. The cells were divided into *C3G* KO and NT groups, and subjected to treatment with hypoxia [7, 9]. The cells were finally randomly divided into NT, *C3G* KO, NT + hypoxia and *C3G* KO + hypoxia groups. The cells were washed with PBS, and harvested at indicated time points respectively for experimental analysis by RT-PCR (reverse transcription-polymerase chain reaction), Western immunoblot, MTT [3-(4,5)-dimethylthiazolium(-z-y1)-3,5-di-phenyltetrazoliumromide] assay and flow cytometry.

RT-PCR analysis for C3G mRNA

Total RNA was purified, quantified and reversely transcribed into cDNA as described previously [7, 9]. The generated cDNA was amplified using specific primers of rat *C3G* and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) genes respectively (Table 1). RT-PCR products were then analyzed.

Western immunoblot experimental analysis for C3G, p-ERK1/2, Bcl-2 and Bax proteins

Protein isolation and its concentration measurement, electrophoretic separation and membrane transferring were similar as previously described [7, 8]. After adding anti-rat *C3G*, p-ERK1/2, Bcl-2, Bax, GAPDH rabbit or mouse primary antibodies respectively, the membrane was incubated overnight, and then incubated with anti-rabbit or anti-mouse second antibody. The intensity of immunoreactive band was then determined [7, 8]. The *C3G* / GAPDH, p-ERK1/2 / GAPDH, Bcl-2 / GAPDH and Bax / GAPDH represented *C3G*, p-ERK1/2, Bcl-2 and Bax protein expression levels respectively.

MTT assay for cell viability in vitro

At the designated time points (24 h, 72 h) after infection, MTT working solution was added to cells in each well plate. Then subsequent performances were similar as described previously [7, 9]. The cell proliferative rate was counted as (absorbance at 72 h – absorbance at 24 h) / absorbance at 24 h, and expressed as a percentage.

Flow cytometry analysis for cell apoptosis in vitro

Seventy-two hours after infection, the apoptotic and necrotic cells were labeled respectively with annexin V fluorescein isothiocyanate (FITC) and propidium iodide (PI). The labeled cells were then sorted by flow cytometry as described previously [7, 9]. The cell apoptotic rates were calculated as the percentage of the number of the annexin V FITC positive and PI negative cells over the number of the total cells.

Myocardial infarction model and experimental protocol

Sixty Sprague-Dawley adult male rats (body weight (BW), 267.1 ± 19.2 g) were divided randomly into sham-operation (sham) + NT (n = 15), Sham + *C3G* KO (n = 15), MI + NT (n = 15) and MI + *C3G* KO (n = 15) groups respectively. MI model and sham-operation were established as described previously [8]. After establishment of MI model and sham-operation, surviving rats were as follows: in sham + NT CRISPR/Cas9 (n = 14), sham + *C3G* CRISPR/Cas9 (n = 13), MI + NT CRISPR/Cas9 (n = 11) and MI + *C3G* CRISPR/Cas9 (n = 10) groups. For each injection point, *C3G* KO or NT lentiviruses (25 μ l) were then injected to myocardium at the following points; 3 points located in the infarcted central zones, and another 8 points in the border zones in each rat with MI or at the corresponding injection points in each reference rat in the above 4 groups respectively (Fig. 3(d)). The chest was then closed, and the rats were treated as described previously [8]. The animals were supplied by the Laboratory Animal Center of Chongqing Medical University, Chongqing, China. All the animal experiments were approved by our institute laboratory animal committee.

Echocardiographic measurements

Transthoracic doppler echocardiographic study was performed as described previously [8]. One week and 12 weeks after treatment with *C3G* KO or NT lentiviruses, the number of surviving rats were shown in each group (Table 2). Interventricular septal thickness (IVST), left ventricle (LV) posterior wall thickness (LVPWT), end-diastolic diameter (LVEDD) and ejection fraction (LVEF) were measured from the M-mode tracings of echocardiography Vivid 7 with a 3.5-MHz sector scan probe (General Electric Medical Systems, Milwaukee, WI, USA). The sonographer, who collected the data, was kept unaware of which treatments the rats were assigned to.

Table 2
Cardiac structure and function

Group	LV/BW (mg/g)	Infarct size (%)	LVEDD (mm)	LVPWT (mm)	LVEF (%)
Sham + NT 1w (n = 13)			5.70 ± 0.60	1.82 ± 0.34	73.63 ± 6.52
Sham + <i>C3G</i> KO 1w (n = 12)			5.64 ± 0.46	1.84 ± 0.37	75.24 ± 7.01
MI + NT 1w (n = 10)	2.00 ± 0.22	0	6.12 ± 0.78	1.79 ± 0.30	69.58 ± 5.89
MI + <i>C3G</i> KO 1w (n = 9)	1.97 ± 0.18	0	6.07 ± 0.72	1.81 ± 0.32	70.91 ± 6.18
Sham + NT 12w (n = 12)	1.63 ± 0.12 ^{*†}	39.13 ± 1.40 ^{*†}	6.20 ± 0.79	0.32	73.46 ± 6.74
Sham + <i>C3G</i> KO 12w (n = 12)	1.80 ± 0.15 ^{*†‡}	38.00 ± 1.20 ^{*†}	5.93 ± 0.71	1.85 ± 0.39	77.39 ± 7.21
MI + NT 12w (n = 8)			7.90 ± 0.99 ^{*†§}	1.88 ± 0.43	43.15 ± 5.38 ^{*†§}
MI + <i>C3G</i> KO 12w (n = 9)			6.94 ± 0.82 ^{*†‡§}	1.65 ± 0.20	49.23 ± 5.56 ^{*†‡§}
				1.73 ± 0.27	

Data are expressed as the mean ± S.D. Note. LV/BW ratio: left ventricular weight / body weight; LVEDD: left ventricular end-diastolic diameter; LVPWT: left ventricular posterior wall thickness; IVST: interventricular septal thickness; LVEF: left ventricular ejection fraction; Sham: sham operation; MI: myocardial infarction; NT: non-target knockout recombinant lentivirus; *C3G* KO: *C3G* knockout recombinant lentivirus. * $P \leq 0.05$ versus Sham + NT group; † $P \leq 0.05$ versus Sham + *C3G* KO group; ‡ $P \leq 0.05$ versus MI + NT group; § $P \leq 0.05$ versus 1w group.

Histology and collagen volume fraction examination

Twelve weeks after sham-operation and MI, BW was measured again; all rats were then euthanized after the echocardiography. The heart was excised rapidly, and the LV was separated from other parts of the heart and weighed. The LV/BW ratio was calculated. Histological slice was obtained and stained with hematoxylin and eosin and picosirius red respectively. Then infarction size and collagen volume fraction (CVF) were measured as described previously [8]. The myocardium in remote zone around the infarcted lesion scar in LV was frozen in liquid nitrogen, and stored in the - 80°C freezer for Western immunoblot examination.

Detection of cardiomyocytic apoptosis in myocardium

According to the instruction of TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) in situ cell death detection kit (No. 11684817910, F. Hoffmann-La Roche Co. Ltd., CH-4070 Basel, Switzerland), the slice was incubated in TUNEL reaction mixture and converter-peroxidase. After

the incubation period, the slice was added with diaminobenzidine substrate. The cardiomyocyte with brown nucleus was defined as apoptotic cell, while the cell with blue nucleus was defined as normal cell. The apoptotic rate was calculated as the ratio of apoptotic to total cell count of cardiomyocytes in each field, and an averaged apoptotic rate was calculated [10].

Statistical analysis

Data is presented as mean \pm standard deviation (SD). All analyses were performed using the SPSS22.0 statistical software. The statistical significance between the groups was evaluated by one-way ANOVA, then, in case of significance, by a two-side Tukey test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

Results

C3G was deleted in vitro cardiac myoblastic and fibroblastic cell lines

C3G mRNA and protein *in vitro* cardiac myoblastic and fibroblastic cell lines were deleted in C3G KO and C3G KO + Hypoxia groups (All $P < 0.05$, Fig. 1(a, b), 2(a, b)). C3G mRNA and protein were diminished in NT + Hypoxia group as compared with NT group (All $P < 0.05$, Fig. 1(a, b), 2(a, b)).

After deletion of C3G, decreased p-ERK1/2, Bcl-2 and increased Bax proteins were found in vitro cardiac myoblastic and fibroblastic cell lines

Decreased p-ERK1/2 and Bcl-2 and increased Bax protein expression in vitro cardiac myoblastic and fibroblastic cell lines were detected after hypoxia both in C3G KO and NT groups (All $P < 0.05$, Fig. 1(b), 2(b)), while lower Bcl-2 and p-ERK1/2 and higher Bax protein expression in the cardiac myoblastic and fibroblastic cell lines were found in C3G KO group than in NT group, either treated with or without hypoxia (All $P < 0.05$, Fig. 1(b), 2(b)).

After deletion of C3G, reduced cell proliferation and elevated apoptosis were confirmed in vitro cardiac myoblastic and fibroblastic cell lines

Hypoxia inhibited cell proliferation and promoted apoptosis *in vitro* cardiac myoblastic and fibroblastic cell lines (All $P < 0.05$), as shown in Fig. 1(c, d) and 2(c, d). C3G knockout group, in addition, exhibited reduction in number of viable cells and an elevated number of apoptotic cells, which were either treated with or without hypoxia (All $P < 0.05$, Fig. 1(c, d), 2(c, d)).

Knockdown of C3G led to decreased p-ERK1/2, Bcl-2 and increased Bax protein expression in surviving myocardium after MI

Twelve weeks after MI, the increase in C3G, p-ERK1/2 and Bcl-2 and decline in Bax protein expression in the surviving myocardium were documented in MI + NT and MI + C3G KO groups as compared to Sham +

NT and Sham + *C3G* KO groups respectively (All $P < 0.05$, Fig. 3(a)). However, there were decrease in *C3G*, *Bcl-2* and p-ERK1/2 and increase in *Bax* protein expression in myocardium in Sham + *C3G* KO and MI + *C3G* KO groups as compared to Sham + NT and MI + NT groups respectively (All $P < 0.05$, Fig. 3(a)).

Knockdown of C3G caused reduction of cardiomyocytic apoptosis and collagen in surviving myocardium after MI

There were increase in CVF and cardiomyocytic apoptosis in the surviving myocardium in MI + NT and MI + *C3G* KO groups as compared to Sham + NT and Sham + *C3G* KO groups respectively (All $P < 0.05$, Fig. 3(b, c)), while the CVF and rate of cardiomyocytic apoptosis in the myocardium in MI + *C3G* KO group were less than that in MI + NT group (Both $P < 0.05$, Fig. 3(b, c)).

Knockdown of C3G improved cardiac structural remodeling and function after MI

LVEDD was increased, while LV/BW ratio and LVEF were decreased in MI + NT and MI + *C3G* KO groups as compared to Sham + NT and Sham + *C3G* KO groups 12 weeks after MI, respectively (All $P < 0.05$, Table 2); While smaller LVEDD and elevated LV/BW ratio and LVEF were found in MI + *C3G* KO group compared to MI + NT group (All $P < 0.05$, Table 2).

Discussion

We demonstrated that cardiomyocytic apoptosis, collagen deposition in the surviving myocardium after MI and LV end-diastolic diameter were decreased; While LV weight / body weight ratio and LVEF were increased with *C3G* knockout lentiviral treatment via down-regulation of pro-survival molecules including *C3G*, p-ERK1/2 and *Bcl-2*, and up-regulation of pro-apoptotic *Bax* expression level in the surviving myocardium. However, in our study, knockout of *C3G* was discovered to delete *C3G* and diminish cellular proliferation *in vitro* cardiac myocytic and fibroblastic cell lines respectively via down-regulation of p-ERK1/2 and *Bcl-2*, and up-regulation of *Bax* protein expression. Knockdown of pro-survival *C3G* in myocardium may mitigate surviving myocardial remodeling after MI, possibly via down-regulation of p-ERK1/2, *Bcl-2* and up-regulation of *Bax* *in vivo* cardiac myocytes and fibroblasts in the surviving myocardium.

With the reparative fibrosis of infarcted myocardium, the remodeling of the surviving myocardium in the border and remote zones around the infarcted lesion is associated with detrimental processes of LV function and poor prognosis. Terminally differentiated cardiomyocyte *in vivo* makes up the most ($\approx 90\%$) of cardiac mass, although it is usually unable to proliferate. Cardiac fibroblast constitutes the majority ($\approx 70\%$) of cells in normal cardiac muscle, because it can proliferate [1]. Therefore, the over-proliferated cardiac fibroblasts are very important for the progressive myocardial remodeling after MI [1].

The surviving myocardial remodeling results from global activation of sympathetic nervous and renin-angiotensin-aldosterone systems (RAAS) in the compensatory responses induced by MI [11, 12]. After MI,

the activated autonomic nervous system and RAAS have been proved to promote cell survival both *in vivo* cardiomyocytes and cardiac fibroblasts via up-regulation of pro-survival gene expression [13, 14]. The over-activation of sympathetic nervous system and RAAS result in ongoing cardiac fibroblast proliferation, myocardial fibrosis, and consequently increase the apoptosis *in vivo* cardiomyocytes, leading to the progressive surviving myocardial remodeling after MI [1].

In contrast, experimental studies have confirmed that globally non-cardiac selective blockage of the activated sympathetic nervous system and RAAS is able to relieve apoptosis of cardiomyocytes and other harmful process in the surviving myocardium after MI [15], although inhibition of the activated autonomic nervous system and RAAS is considered to result in apoptosis *in vivo* cardiomyocytes and cardiac fibroblasts via up-regulation of pro-apoptotic gene expression [15].

A previous study has proved that cardiomyocyte-specific knockout of a pro-apoptotic gene attenuated myocardial remodeling induced by pressure overload or MI [2]; However, systemic knockout of the same pro-apoptotic gene can augment LV remodeling process [2].

Integrin pathway links extracellular matrix substances to cytoskeleton in cells, and senses mechanical stretch, and translates it into intracellular biochemical signaling [3, 16]. Cardiomyocyte-specific deletion of pro-survival integrin $\beta 1$ subunit and its downstream molecules such as FAK may increase apoptosis *in vivo* cardiomyocytes and aggravate cardiomyopathy induced by MI, isoproterenol or pressure overload [4, 5]. Knockout of cardiomyocyte-specific pro-survival FAK or integrin-linked kinase (ILK) even spontaneously results in dilated cardiomyopathy with reduced LV function [17, 18]. However, a previous study has demonstrated that globally systemic inhibition of pro-survival FAK attenuated cardiomyopathy and preserved cardiac function in post-MI [19]. Therefore, knockdown of the integrin pathway pro-survival component C3G in myocardium may have a protective effect against myocardial remodeling after MI.

Pro-survival C3G protein is a family member of guanine nucleotide exchange factor in the integrin pathway. After stimulation by signals through integrins, C3G binds with N-terminal Src homology 3 domain of its upstream adaptor CrkL, and subsequently activates its downstream GTPases such as Rac1 [20]. On the basis of existing evidences, pro-survival C3G can inhibit cell apoptosis *in vitro* cardiomyocytes via up-regulation of its downstream pro-survival AKT, p-ERK1/2, Bcl-2 and down-regulation of pro-apoptotic Bax [7, 9]. In addition, our current experimental study has replenished that knockout of pro-survival C3G by a specific knockout lentivirus can promote cell apoptosis *in vitro* cardiac fibroblastic cell line via down-regulation of p-ERK1/2, Bcl-2, and up-regulation of Bax. A previous study has confirmed that pro-survival expression of C3G, p-ERK1/2 and Bcl-2 were up-regulated, while pro-apoptotic Bax expression was down-regulated in the surviving myocardium after MI [8]. These molecular expression changes possibly resulted from the global activation of sympathetic nervous system and RAAS induced by MI. However, for the first time, we have documented that knockdown of pro-survival C3G in myocardium mitigated cardiomyocytic apoptosis, myocardial fibrosis and remodeling around the infarcted lesion, possibly via down-regulation of pro-survival p-ERK1/2, Bcl-2, and up-regulation of pro-apoptotic Bax mainly *in vivo* cardiac fibroblasts in the surviving myocardium.

The underlying mechanisms for the mitigation of the surviving myocardial remodeling after MI by knockdown of pro-survival C3G in myocardium are as follows: Cardiac fibroblast *in vivo* may play a major role in cell survival *in vivo* cardiomyocytes after MI [1]; While the proliferative cardiac fibroblasts *in vivo* may be more susceptible to the treatment with C3G knockdown lentivirus than non-proliferative cardiomyocytes *in vivo*. Knockdown of pro-survival C3G may mainly depress the proliferation of cardiac fibroblasts in the myocardium, and alleviate myocardial fibrosis, and consequently result in inhibition of cardiomyocytic apoptosis and myocardial remodeling after MI, although knockdown of C3G may lead to cellular apoptosis both *in vivo* cardiac fibroblasts and myocytes in the surviving myocardium. Appropriate-intensity transient knockdown of pro-survival C3G, which may mainly inhibit cardiac fibroblast proliferation, could be developed as a novel potential therapeutic measure to relieve the surviving myocardial remodeling, fibrosis, ischemic cardiomyopathy and cardiac dysfunction. Moreover, we have used experimental results *in vitro* H9C2 cardiac myoblasts to interpret mechanisms for cardiac structural phenotypic changes *in vivo*. As considering that the H9C2 cell has some different phenotypes from adult primary cardiomyocyte, the conclusions made in this study should be taken cautiously.

In this study, the recombinant lentivirus can knockout C3G *in vitro* cardiac myocytic and fibroblastic cell lines. However, it can not knockout all of C3G in the myocardium, because C3G knockout lentivirus was administered just transiently in the myocardium. Nevertheless, it can knockdown part of C3G in the myocardium and positively affect the myocardial remodeling. The effects of permanently systemic C3G knockout on surviving myocardial remodeling after MI are still unclear. The role of pro-survival C3G in myocardial remodeling remains to be redefined in the future via establishment of animal transgenic model with systemic knockout of C3G.

Conclusion

Our current data demonstrate that knockdown of pro-survival C3G in myocardium mitigates surviving myocardial remodeling after MI, possibly via down-regulation of p-ERK1/2, Bcl-2 and up-regulation of Bax *in vivo* cardiac myocytes and fibroblasts in the surviving myocardium.

Declarations

Acknowledgements Not applicable

Authors' contributions GL provided the concept, administration, supervision, resources and funding for the present study, and validated the data. QD, SH, CL and NT collected the data. QD and GL analyzed the data. QD and GL prepared the figures. GL and JAL drafted, edited and critically reviewed the manuscript. All authors reviewed the final manuscript.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Consent to participate All authors participate and contribute to the research.

Consent to publish All authors have read and approved submission of the manuscript

Ethical approval The study protocol used in the study was approved by the institutional laboratory animal ethics committee of the First Affiliated Hospital of Chongqing Medical University.

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Figures

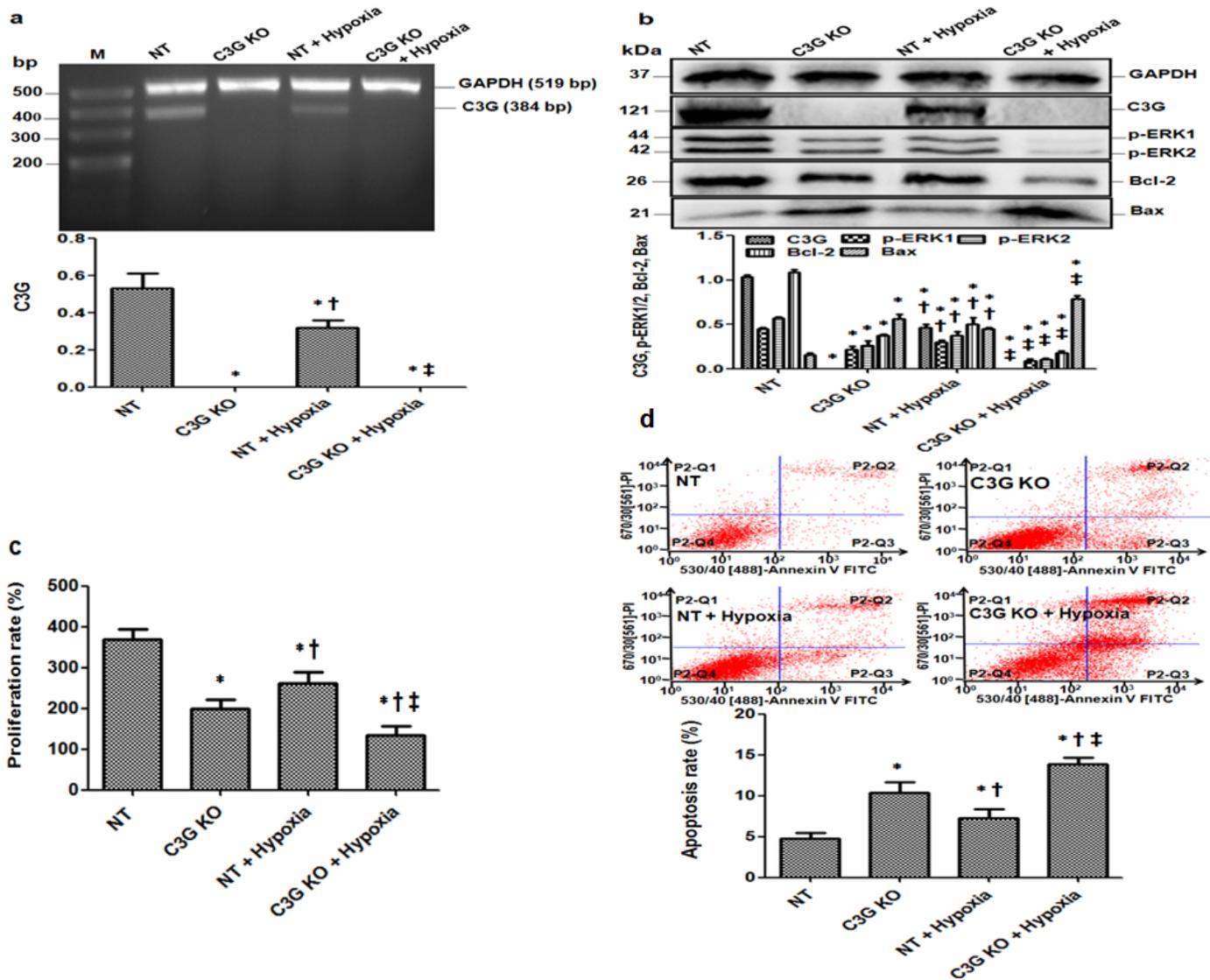


Figure 1

a C3G mRNA was deleted in vitro H9C2 cardiac myoblastic cell line. b After C3G protein was deleted, the decreased p-ERK1/2, Bcl-2 and increased Bax proteins consequently exhibited in the cardiac myoblasts. c Proliferative rate was decreased in the cardiomyoblasts after C3G knockout. d Apoptotic rate was increased in the cardiac myoblasts after C3G knockout. Note NT: Non-target lentivirus; C3G KO: C3G knockout lentivirus. PI: Propidium iodide; Annexin V FITC: Annexin V fluorescein isothiocyanate. * $P \leq 0.05$ versus NT group; † $P \leq 0.05$ versus C3G KO group; ‡ $P \leq 0.05$ versus NT + Hypoxia group. Data were expressed as mean values \pm S.D., $n=3$.

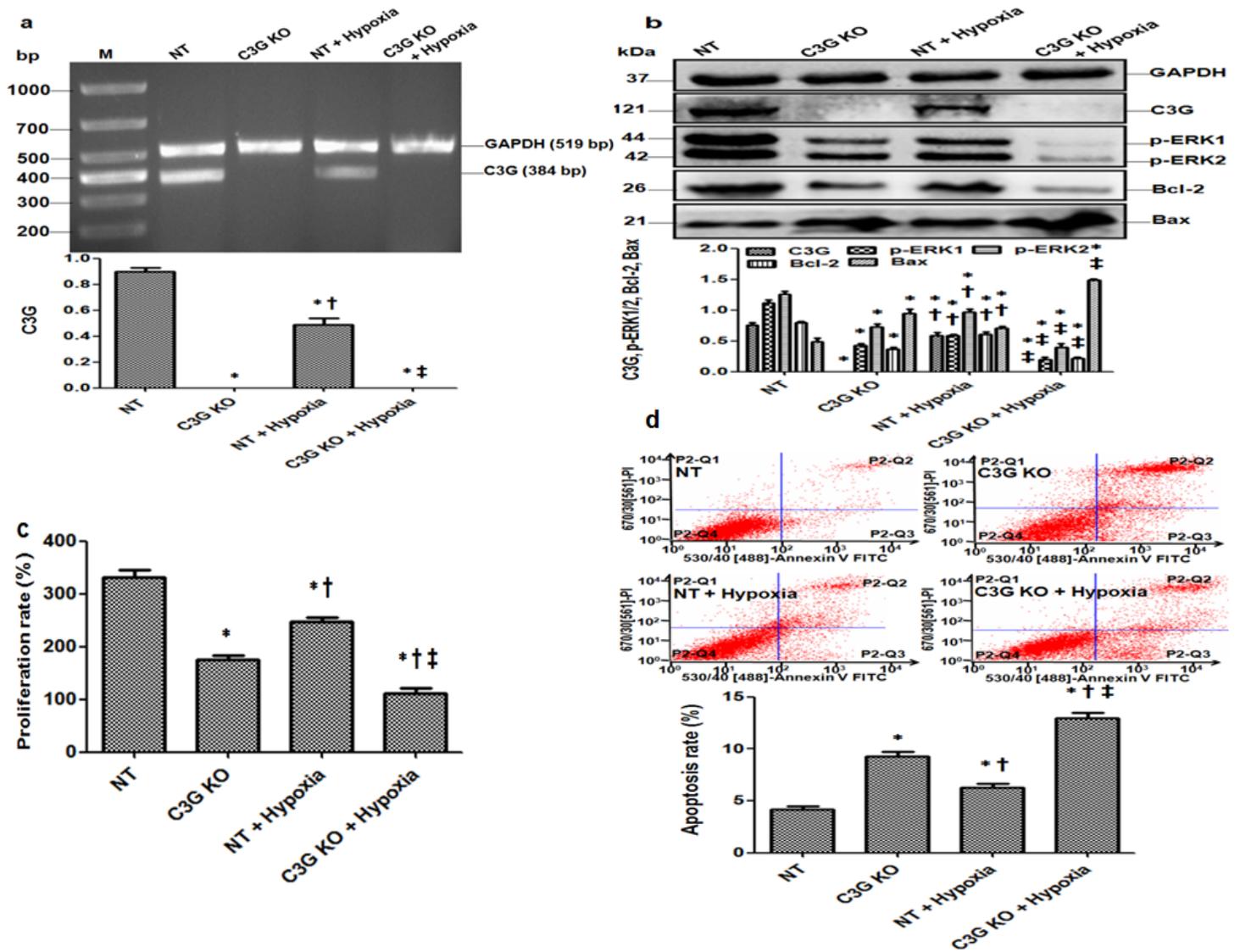


Figure 2

a C3G mRNA was deleted in vitro cardiac fibroblastic cell line. b After C3G protein was deleted, the decreased p-ERK1/2, Bcl-2 and increased Bax proteins consequently exhibited in the cardiac fibroblasts. c Proliferative rate was decreased in the cardiac fibroblasts after C3G knockout. d Apoptotic rate was increased in the cardiac fibroblasts after C3G knockout. Note NT: Non-target lentivirus; C3G KO: C3G knockout lentivirus. PI: Propidium iodide; Annexin V FITC: Annexin V fluorescein isothiocyanate. * $P \leq 0.05$ versus NT; † $P \leq 0.05$ versus C3G KO; ‡ $P \leq 0.05$ versus NT + Hypoxia group. Data were expressed as mean values \pm S.D., n=3.

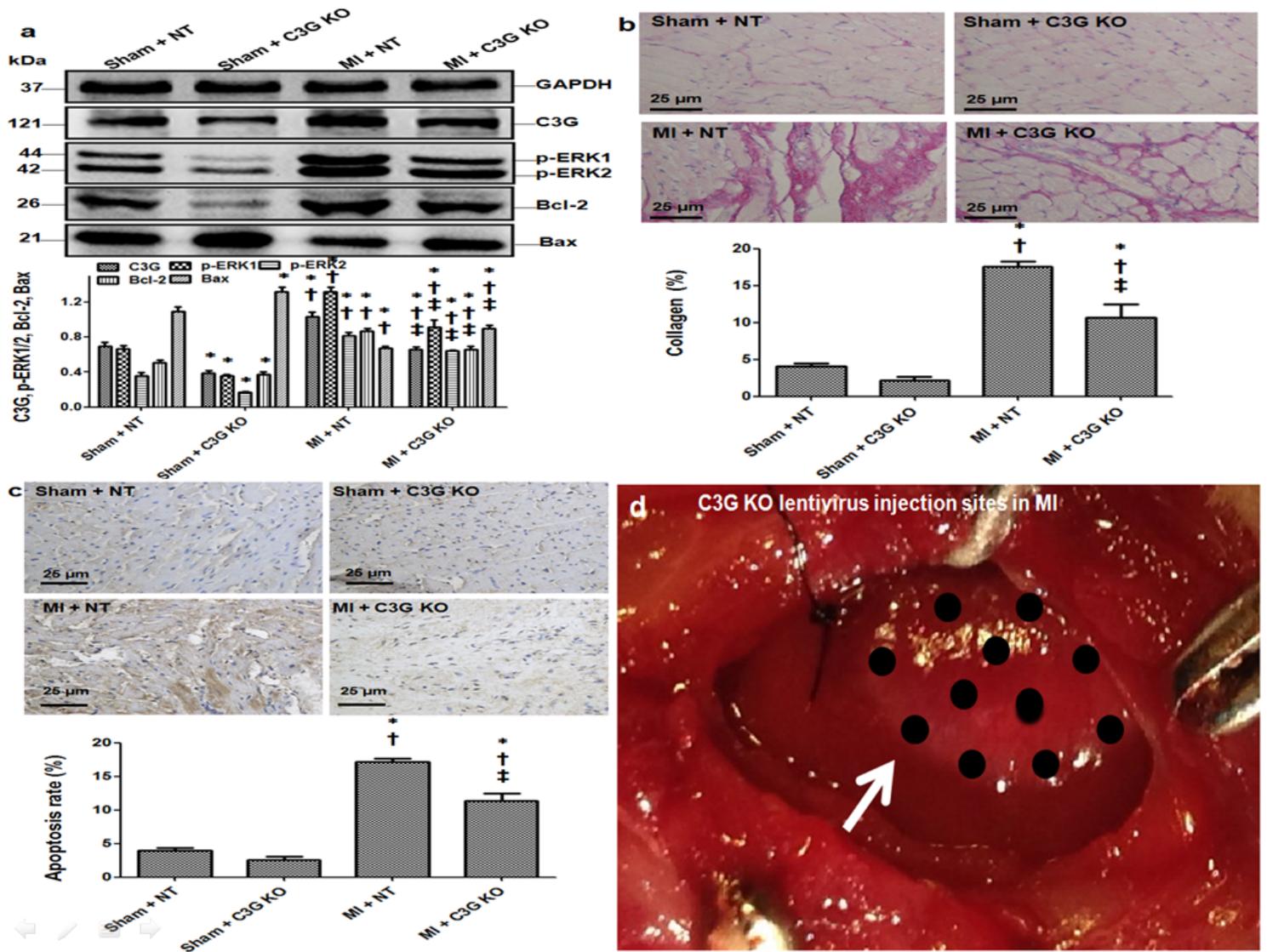


Figure 3

a Increased C3G, p-ERK1/2, Bcl-2 and decreased Bax proteins were detected in the surviving myocardium after MI, while after C3G knockdown, decreased p-ERK1/2, Bcl-2 and increased Bax proteins consequently exhibited in the surviving myocardium 12 weeks after MI. b After C3G knockdown, reduction of collagen volume fraction was examined in the surviving myocardium 12 weeks after MI (x 400). c After C3G knockdown, decreased cardiomyocytic apoptosis was detected in the surviving myocardium 12 weeks after MI (x 400). d C3G knockout lentiviral injection sites in the infarcted myocardial central zone and its border zone. Note Sham: Sham operation; NT: Non-target lentivirus; C3G KO: C3G knockout lentivirus. * $P \leq 0.05$ versus Sham + NT group (n=12); † $P \leq 0.05$ versus Sham + C3G KO group (n=12); ‡ $P \leq 0.05$ versus MI + NT group (n=8). Data were expressed as mean values \pm S.D.