RNA Binding Protein QKI Inhibits the Ischemia/reperfusion-induced Apoptosis in Neonatal Cardiomyocytes

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Key Words

Cardiomyocytes • Ischemia/reperfusion • QKI • Apoptosis • FoxO1

Abstract

Backgrounds: RNA-binding protein QKI is abundantly expressed in the brain and heart. The role of QKI in the nervous system has been well characterized, but its function in cardiac muscle is still poorly understood. The present study was to investigate the role of QKI in ischemia/reperfusion-induced apoptosis in cardiomyocytes. **Methods:** A simulated ischemia/reperfusion model was established in neonatal cardiomyocytes and adult rat heart. After QKI5 or QKI6 was expressed by adenovirus and QKI was knocked down QKI by RNAi in the cardiomyocytes, RT-PCR, western blot and immunofluorescence staining were applied to detect gene expression alterations. Apoptosis was evaluated by PARP degradation, DNA fragmentation (DNA laddering) and flow cytometry. **Results:** Our study demonstrated that both QKI5 and QKI6 were present in cardiomyocytes, while QKI5 expression was greatly inhibited by simulated ischemia/reperfusion. Knocking down endogenous QKI by RNAi enhanced cell susceptibility to apoptosis,

whereas overexpression of either QKI5 or QKI6 suppressed IR-induced apoptosis substantially. The pro-apoptotic transcription factor FoxO1, a potential QKI target, was induced by ischemia/reperfusion at both total amount and nuclear distribution. Accordingly, FOXO1 downstream target genes were negatively affected by the presence of QKI with IR treatment. **Conclusion:** In summary, our study supports that both QKI-5 and 6 are anti-apoptotic proteins in cardiomyocytes, favoring cardiac survival via antagonizing the elevation of some pro-apoptotic factors in cardiac injury.

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Introduction

The *quaking* viable (*qk*^v) mice have been studied for more than 40 years since the allele was first defined by Sidman and his coworkers [1]. The mice carry a spontaneous mutation that affects maturation of the myelinating cells in the central nervous system (CNS), causing extensive tremors and at late stages, tonic type

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seizures. The *qk^v* mutation consists of a 1-megabase deletion containing the promoter and enhancer regions of *qkl* gene. This gene produces several alternative splicing variants and encodes 3 major QKI isoforms, namely QKI-5, -6 and -7 respectively. These isoforms are only different in the carboxy-terminal ends, which determine the nuclear localization of QKI-5 and cytoplasmic localizations of QKI-6 and QKI-7 [2-5]. *In vivo*, QKI are largely present in virtually all of the major organs in adult mice, with the highest levels in the brain, lung and heart [3, 4]. The demyelination phenotype in *quaking* viable (*qk^v*) mice provides a valuable model to address the role of QKI in CNS [5-8].

As one of the signal transduction and activation of RNA (STAR) family members, QKI exerts its function by mediating the upstream kinase signals to directly modulate various RNA metabolism processes, including pre-mRNA splicing, mRNA export, mRNA stability and protein translation [9, 10]. A bipartite consensus sequence NACUAAAY-N₁₋₂₀-UAAY was designated as QKI response element (QRE) and as many as 1,430 new mRNA targets were predicted to be QKI direct targets. It remains a big challenge to validate those regulations by QKI and the functional significance *in vivo* [11].

Among thousands of the potential target genes of QKI, FoxO1 is one of transcription factors that mediate a wide range of cellular responses, from cell death to growth inhibition and glucose utilization [12]. In the heart, FoxO proteins have been shown to be a negative regulator in neonatal heart proliferation and cardiac hypertrophic growth [13, 14]. FoxO family members themselves were also found to be regulated by complicated mechanisms, including phosphorylation, ubiquitination and acetylation. In order to explore the functional role of QKI in the heart, we first observed its expression changes under ischemia/reperfusion (IR), and then we continued to demonstrate its protective role during the injury and its relevance with FoxO1 alterations.

Methods and Materials

Cell Culture

Primary culture of neonatal rat cardiomyocytes was prepared as previously described [15]. In brief, the hearts from 1–3 day-old Sprague-Dawley rats were minced and dissociated with collagenase. Cell suspension was then plated on a 10 cm dish for 1 h, which allows most of the fibroblasts to adhere. The supernatant was then plated onto a new dish (remaining fibroblasts plus cardiomyocytes). The remaining fibroblasts were further suppressed by addition of 5-bromodeoxyuridine

for 24 h. After 3–4 days of incubation, cells were subjected to subsequent treatments.

H9C2 cells, a rat embryo heart-derived myoblast cell line (American Type Culture Collection, ATCC) were cultured with DMEM medium containing 10 % fetal bovine serum, 5 % CO₂, 37 °C.

In vitro ischemia/reperfusion

In neonatal cardiomyocytes (NCMs) and H9C2 cells, ischemia was mimicked by replacing growth medium with HEPES buffer solution (NaCl 137 mM, KCl 2.7 mM, Na₂HPO₄ 8 mM, KH₂PO₄ 1.5 mM, CaCl₂ 0.9 mM, and MgCl₂ 0.5 mM, initial pH: 7.2) and reducing oxygen from 20% to 1 % O₂ while maintaining CO₂ constant at 5 %. Reperfusion was simulated by restoring growth medium and culturing cells in regular incubator with 20 % oxygen [15]. For QKI protein detection, a protocol of 4 h ischemia plus 6 h reperfusion was applied to NCMs. For apoptosis detection, a process of 6 h ischemia plus 6 h reperfusion was applied to NCMs and 9 h ischemia plus 6 h reperfusion were applied to H9C2 cells.

Cardiac IR model

In brief, anesthesia was induced in adult, 200-g SD rat with ketamine (100 g/g), xylazine (20 g/g), and isoflurane (1%). The heart was exposed through a left thoracotomy, and a 7-0 silk suture was placed around the left anterior descending artery (LAD) as it emerged from under the left atrium. The LAD was occluded over a short piece of P-10 tubing for a period of 30 min prior to removal for reperfusion. Ischemia was confirmed by both ST elevation on surface electrocardiogram and visual blanching. Hearts were harvested 4 h after reperfusion. All experiments were performed with approval and under guidelines of the Fourth Military Medical University Institutional Animal Care and Use Committee.

SiRNA design and transfection

The QKI siRNA sequence is designed as the following “CGC CAA CAU UAA AUC ACC AGC CCU U” for sense and “AAG GGC UGG UGA UUU AAU GUU GGC G” for anti-sense. The negative control RNA duplex was synthesized as well: “CGC CAU AAU AAC UCA ACC GCA CCU U” for sense and “AAG GUG CGG UUG AGU UAU UAU GGC G” for anti-sense. Transfection was performed with Lipofectamine 2000 (Invitrogen, USA) following manufacturer’s instructions.

RT-PCR

Total RNA was extracted using TRIzol (Invitrogen) according to the manufacturer’s protocol. DNA was removed by DNase. Two micrograms of RNA were reverse transcribed with Superscript III reverse transcriptase (Invitrogen) using random primers. The cDNA was used for PCR. The expression of β-actin was used as an endogenous reference. The primers are as follows: FOXO1 (forward, 5’-GAG CGT GCC CTA CTT CAAGG-3’; reverse, 5’-TCC TGG CCAGAC TGG AGAGA-3’), β-actin (Forward 5’- AGC CAT GTA CGT AGC CAT CC-3; Reverse, 5’-TCT CAG CTG TGG TGG TGA AG-3’). The PCR products are 270 bp and 501 bp for FOXO1 and β-actin respectively.

Adenovirus Preparation and infection

Full-length human QKI5 cDNA, tagging with 3×Flag, was cloned from human cDNA library and inserted to pIRES2-EGFP (Clontech), which produced the plasmid QKI5-EGFP. Full-length QKI6 cDNA was inserted to pcDNA3.1 (+), producing QKI6 expression plasmid. Recombinant adenoviruses encoding these genes were generated via AdEasy adenovirus construction kit following the manufacturer's instruction. Viral supernatants were clarified by centrifugation and titered by plaque assays. Aliquots of virus were stored frozen at -80°C. Ad-EGFP was used as a control virus.

For adenovirus-mediated protein over-expression, cells were incubated for 2 h with adenovirus at a multiplicity of infection (MOI) of 10 to 50; the virus was removed, and cells were cultured for an additional 48 h in fresh DMEM/M199 containing 10 % fetal bovine serum before subjecting to IR treatment. Under these conditions, infection efficiency usually exceeded 90 %.

Analysis of QKI, FOXO1, PARP, Bim and FasL by Western Blotting

The level of protein expression was analyzed using Western blotting assay. In brief, cells were harvested in ice-cold lysis buffer (Tris 50 mM, NaCl 150mM, NP40 1 %, SOD 0.25 %, EDTA 1mM, NaF 1mM, Na₃VO₃ 1mM, PMSF 1 M, Roche proteinase inhibitor cocktail); the total protein was measured using a Bradford protein assay. The lysates were loaded on SDS-polyacrylamide gel and transferred onto nitrocellulose membranes, which were then immunoblotted with anti-QKI (1:2000, made in our lab), anti-FOXO1 (1:1000, Cell Signaling), anti-PARP (1:1000, Cell Signaling), anti-Bim (1:1000, Cell Signaling) or anti-FasL (1:1000, Cell Signaling). Quantification of Western blots was obtained by multiplying the area and intensity of each band using ImageJ software. The graphs shown in Fig. 1 were normalized to Tubulin as the loading control.

Immunofluorescence analysis

For immunofluorescence analysis, cells were grown on coverslips. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS after indicated treatment. Cells were then incubated with mouse anti-QKI monoclonal antibody (1:200) or rabbit anti-FOXO1 antibody (Cell Signaling, 1:100) as a primary antibody overnight at 4 °C in a humidified chamber. Excess antibody was removed by washing the cover slips 3 times with PBS. Cells, which were protected from light, were then incubated with FITC-conjugated rabbit anti-mouse IgG (1: 600 for QKI detection) or CY3-conjugated goat anti-rabbit IgG (1:600 for FOXO1 detection) for 2 h at room temperature. The primary and secondary antibodies were diluted in 5% FBS-PBS for use. After washing 3 times with PBS, cells were mounted upside-down in Hoechst 33258-containing anti-quenching buffer on microscope slides and sealed with nail polish. The cells were examined and photographed under a fluorescence microscope.

Apoptosis Assays

Flowcytometry. Fluorescein isothiocyanate (FITC)-

conjugated Annexin V and propidium iodide (PI) (Annexin-V-FUOS, Boehringer Mannheim, Mannheim, Germany) were used to identify apoptotic cells [15]. This assay discriminates between intact (FITC-/PI-), early apoptotic (FITC+/PI-), and late apoptotic (FITC+/PI+) cells. Comparative experiments were performed at the same time by bivariate flow cytometry using a FACScan (BD) and analyzed with CellQuest (BD Biosciences, Franklin Lakes, NJ) software on data obtained from the cell population.

PARP degradation. PARP, a 116 kDa nuclear poly (ADP-ribose) polymerase, is involved in DNA repair in response to environmental stress. This protein is one of the main cleavage targets of caspase-3 *in vivo*. The cleavage separates the PARP amino-terminal DNA binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa), which facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis [16]. PARP degradation was determined by Western blotting as described above. The apoptotic level was indicated by intensity of 89 kDa degraded PARP band normalized by α -tubulin (loading control).

DNA laddering. DNA fragmentation is a hallmark of apoptosis. For assessing DNA fragmentation (DNA ladder), NCMs were trypsinized and washed with PBS, and then incubated with mammalian cell lysis buffer (0.1% SDS, 10 mmol/L EDTA, 10 mmol/L Tris, pH=8.0) containing 20 μ g/ml heat-inactivated RNase A at 37°C for 1 h, and with 0.5 mg/ml proteinase K for another 60 min. Cell lysates were incubated at 50°C for 2 h and isopropanol was added. After ethanol precipitation, 8 μ g of DNA sample was separated by electrophoresis on 2% agarose gel containing ethidium bromide (0.5 μ g/ml). DNA ladder formation, a "hallmark" for cell apoptosis, was visualized under an ultraviolet light and photographed for permanent records.

Data analysis and statistics

All data are presented as mean $\pm$ s.e.m. Analysis of variance (ANOVA) for random measures was performed followed by post hoc Fisher test to determine statistically significant differences. $P < 0.05$ was considered significant.

Results

QKI expression in cardiomyocytes

Previously, QKI-5, -6 and -7 transcripts were detected in the heart by Northern blotting and QKI-5 and -6 proteins were detected by Western blotting [3]. Our study used a pan-QKI polyclonal antibody to determine QKI expression in the neonatal cardiomyocytes (NCMs), and two bands at 40-kDa and 38-kDa sites corresponding to QKI-5 and QKI-6 were observed (Fig. 1A). Quantification data were shown in Figure 1B. Immunofluorescence observation revealed that QKI was mainly located in the nucleus with weak signal in the cytoplasm (Fig. 1C). Hence, QKI-5 was the dominant

Fig. 1. QKI expression in neonatal cardiomyocytes (NCMs) under simulated ischemia/reperfusion. The cells were deprived of growth medium by a HEPES buffered solution, of oxygen by a tri-gas incubator with 1 % O₂ or of both (ischemia) for indicated hours. Reperfusion was mimicked by restoring growth medium and oxygen in a regular incubator with 5 % CO₂ and 95 % air. (A) Total lysates were applied to western blotting probed with antibody against QKI, and a sample blot is presented. (B) Bar graph is quantifications of QKI5 and QKI6 expressions. The relative protein contents were represented by folds of the densitometry of control (no treatment). The data, from 3 experiments, were expressed by mean±S.D. (C) NCMs with no treatment or ischemia/reperfusion were labeled with monoclonal antibody against QKI (green) and a nuclear-specific fluorescence dye Hoechst33258 (blue). Representative images are shown. Scale bar: 50 μm. ** (QKI5) $P<0.01$ vs control (no treatment); ## (QKI6) $P<0.01$ vs control.

Fig. 2. QKI expression in myocardium under ischemia/reperfusion *in vivo*. The hearts of adult SD rats was exposed through a left thoracotomy, and a 7-0 silk suture was placed around the left anterior descending artery (LAD) as it emerged from under the left atrium. The LAD was occluded over a short piece of P-10 tubing for a period of 30 min prior to removal for reperfusion. Hearts were harvested 4 h after reperfusion. (A) Total lysates were applied to western blotting probed with antibody against QKI, and a sample blot is presented. (B) Bar graph is quantifications of QKI5 and QKI6 expressions. ** $P<0.01$ vs Sham control.

isoform in NCMs in terms of the supposed subcellular localization of QKI isoforms [2-5].

Simulated IR and QKI expression

QKI expression under simulated IR was investigated by Western blotting and immunofluorescence (Fig. 1A and 1C). Deprivation of growth medium alone for 4 h could inhibit QKI-5 expression by 41.3 ± 7.6 % in NCMs; depriving oxygen alone for 4 h could reduce QKI-5 expression up to 93.2 ± 2.2 %. The combination (medium and oxygen withdrawal) for 4 h suppressed QKI-5 expression by 96.4 ± 0.6 %. Therefore, inhibition of QKI5 expression is mainly a consequence of oxygen supply withdrawal. Restoration of culture medium and oxygen for 6 h not only failed to rescue, but also led to further reduction of QKI-5 expression (Fig. 1A and 1B). The reduction was also seen in immunofluorescence assay (Fig. 1C). In order to validate the above *in-vitro* observations, we set up the rat cardiac IR model to simulate the *in-vivo* condition, expectedly the adult heart

samples showed the similar reductions of QKI under IR treatment as shown in Fig. 2,

QKI and ischemia/reperfusion-induced apoptosis

Since QKI5 was nearly cleared by IR, it is intriguing to know the possible relationship between this clearance

and ischemia/reperfusion-induced apoptosis. We next investigated the IR-induced apoptosis with premise of QKI clearance. RNA interference (RNAi) against QKI was applied to see the effect of “QKI clearance” on IR-induced apoptosis in cardiac myoblast H9C2 cells. The interference effect was confirmed by RT-PCR (Fig. 3A).

Fig. 4. The effects of QKI 5 and QKI6 over-expression on ischemia/reperfusion-induced apoptosis indicated by PARP degradation and DNA fragmentation (DNA laddering) in neonatal cardiomyocytes (NCMs). NCMs were treated with 6 h ischemia plus 6 h reperfusion 48 h after infection of adenoviruses expressing QKI -5 or -6. Ad-EGFP was used as control virus corresponding the Ad-QKI-5 or -QKI6. (A) The cells were harvested for detecting PARP degradation by Western blotting, and the sample blots are presented (B) Quantitative analysis of the relative values of 89 kDa PARP was determined by densitometry analysis of Western blots. Data are represented as fold change of the value of control cells (treated with Ad-EGFP only). The data, from 3 experiments, are expressed by mean±S.D.. ** P <0.01. (C) The effects of QKI 5 and QKI6 over-expression on ischemia/reperfusion-induced apoptosis were indicated by DNA fragmentation (DNA laddering) in neonatal cardiomyocytes (NCMs). NCMs were treated as above. The cells were also harvested for DNA fragmentation test on agarose gel. DNA laddering, and sample figure is presented.

Then, the above cells were treated with an optimized apoptosis condition, as 9 h of simulated ischemia plus 6 h reperfusion. Flow cytometry experiment showed that the apoptosis rate was greatly enhanced after knocking down QKI, with the rate of $54.9 \pm 3.5\%$ compared to $33.2 \pm 2.4\%$ in the negative control one (Fig. 3B). We did not repeat the experiment in NCMs because of the failure to induce an effective RNAi in NCMs due to the low transfection efficiency.

On the other hand, adenovirus overexpressing QKI5 or QKI6 was employed to maintain QKI expression under simulated IR in NCMs. In this episode of experiment, treatment with 6 h ischemia plus 6 h reperfusion produced

the more satisfactory apoptosis in NCMs, determined by PARP cleavage (Fig. 4A) and DNA fragmentation (Fig. 4C). Higher level of cleaved PARP (89kDa), compared to native one (116 kDa), is an important hallmark of apoptotic activity. The cells with IR treatment showed an obvious cleaved PARP band, which was greatly inhibited by QKI5 or QKI6 overexpression (Fig. 4A). Densitometry indicated that Ad-QKI5 and Ad-QKI6 inhibited the PARP cleavage by $67.6 \pm 4.5\%$ and $68.2 \pm 3.3\%$ (Fig. 4B). Furthermore, DNA laddering showed that IR induced obvious DNA fragmentation in NCMs, which was also significantly suppressed by QKI 5 or QKI 6 over-expression (Fig. 4C). These data

Fig. 5. FOXO1 expression in myocardium under ischemia/reperfusion *in vivo*. The hearts of adult SD rats were treated as described in Figure 2. (A) Total lysates were determined by Western blotting probed with antibody against FOXO1, and a sample blot is presented. (B) Bar graph is quantification of FOXO1 expression. ** $P < 0.01$ vs Sham control.

Fig. 6. Effects of QKI siRNA on FOXO1 expression in cardiac myoblast H9C2 cells under simulated ischemic/reperfusion. Negative control (ncRNA) or small interference RNA against QKI (siRNA) was transfected into cardiac myoblast H9C2 cells 48 h before the cells were subjected to simulated ischemia/reperfusion. (A) Total lysates were detected by Western blotting probed with antibody against FOXO1, and a sample blot is presented. (B) Bar graph is the quantification of FOXO1 expression. ** $P < 0.01$.

collectively support that QKI5 and QKI6 are potent anti-apoptotic proteins in NCMs.

Regulation of FOXO1 by QKI

Following the above verified anti-apoptotic roles of QKI5 and QKI6 in cardiomyocytes, it was valuable to explore the underlying mechanisms. The pro-apoptotic transcriptional factor FoxO1, a predicted target of QKI, was considered as a candidate. The endogenous level of FoxO1 was very low under normal conditions both in adult myocardium and cultured NCMs, but it was greatly induced by ischemia/reperfusion both *in vivo* and *in vitro* (Fig. 5A, 6A).

“QKI clearance” by siRNA against QKI did not change FOXO1 expression in cells without IR, but enhanced IR-induced FOXO1 elevation (Fig. 6A, B). Adeno-QKI5 or -QKI6 did not change the FoxO1 expression in cells under normal condition. However, they almost completely abrogated FoxO1 up-regulation under ischemia(6h)/reperfusion(6h) stimuli (Fig. 7A and 7B). This inhibition took effects at both RNA level and protein level.

As known before, several apoptotic proteins, including Bim and FasL, are transcriptionally activated by FoxO1 under apoptotic conditions [17], especially in IR [18], it was therefore necessary to observe their alterations too. As expected, both Bim and FasL levels were repressed by over-expressed QKI-5 or -6 under IR treatments, which were consistent with FoxO1 activation status (Fig. 7A). In addition, Immunofluorescence analysis was applied to observe sub-cellular distribution of FoxO1, since nuclear trafficking is critical for FoxO1 activation (Fig. 8). FoxO1 was only slightly stained in the cytoplasm and nucleus under normal conditions, which was not affected by over-expressing Ad-QKI5, QKI-6 and Ad-EGFP. Following ischemia (6 h)/reperfusion (6 h), FoxO1 staining in the nucleus was greatly intensified in the control Ad-EGFP cells, whereas it was significantly suppressed when being infected with Ad-QKI5 or Ad-QKI6 (Fig. 8). From these data, it was very likely that FoxO1 was one of target genes inhibited by QKI, participating in IR-induced cardiac myocyte apoptosis.

Discussion

Our observations here provide the first evidence that RNA-binding protein QKI-5 and -6 exert the anti-apoptotic roles in cardiomyocytes under IR treatment,

Fig. 7. The regulation of FOXO1 mRNA expression and its apoptosis related targets by QKIs. (A) The neonatal cardiomyocytes (NCMs) were treated as in Figure 2. Representative figures are presented showing FOXO1 transcripts detected by RT-PCR. Bar graph is quantification of FOXO1 mRNA level (B). ** $P < 0.01$ vs control (treated with Ad-EGFP only). (C) Representative figures are presented showing FOXO1, Bim and FasL detected by Western blot. (D) Bar graph shows quantifications of FOXO1, Bim and FasL protein level. # $P < 0.05$, ## $P < 0.01$ vs control (treated with Ad-EGFP only); * $P < 0.05$, ** $P < 0.01$ vs (Ad-EGFP+IR) group.

possibly via inhibition of pro-apoptotic transcriptional factor FoxO1 either directly or indirectly. QKI and FoxO1 may work as yin-yang partners determining the anti- and pro-apoptotic homeostasis in the heart. Under severe IR injury conditions, QKI reduction facilitates FoxO1 up-regulation and activation, and therefore contributes to the apoptosis observed.

IR damage in the myocardium involves an imbalance between detrimental and self-protective factors [19, 20]. Hence, identification and characterization of the regulating

molecule in IR injury are valuable for establishing strategies to maximize the benefit of myocardial reperfusion. Previous findings of QKI in the vessel system have established its important role in early development [21-23], but its importance in adult heart remains unknown. We initially observed QKI expression under normal survival as well as IR circumstances *in vivo* and *in vitro*. Our study here provides the first evidence that QKI-5 and QKI-6 have a protective role in IR-induced cell apoptosis.

Fig. 8. The effects of QKI-5 and QKI-6 over-expression on the subcellular localization of FoxO1. NCMs with Ad-QKI5-3XFlag and Ad-QKI6 infections were subjected to simulated ischemia/reperfusion, and the cells were labeled with antibody against FOXO1 using immunofluorescence. Ad-EGFP was used as a control virus. Representative figures are shown probed FOXO1 using immunofluorescence. IR, ischemia/reperfusion. Scale bar: 30 μ m.

As to how QKI-5 and QKI-6 protect the cell from apoptosis. FOXO1 might be an essential mediator. FoxO1 is a well known negative regulator for cardiac hypertrophy and proliferation [13, 14]. Under normal survival conditions, FoxO1 phosphorylated by Akt was sequestered and even degraded in the cytoplasm [24, 25]. Upon stress stimuli, such as IR treatment, inhibition of AKT or induction of P38 kinase tends to transport FoxO1 to the nucleus and reactivate its downstream pro-apoptotic and cell cycle arrest responses [26-29]. Here in our system, we observed not only an up-regulation of FoxO1 in the nucleus (dephosphorylated form), but also a dramatic increase in total FoxO1 expression upon IR treatment in cardiomyocytes, highly indicating its pro-apoptotic role during the process, which is consistent with a study by Sengupta [30]. It is worthy to note that in the above IR experiment, FoxO1 changes was profoundly affected by presence of QKI. Overexpression of QKI-5 or -6 effectively blocked FoxO1 elevations, whereas knocking down QKI enhanced its up-regulation. Consequently it is plausible to propose that the anti-apoptotic effects of QKI are partially mediated by its inhibition on FoxO1 expression changes under IR condition.

Previous studies have shown that GLD-1, a homologue of QKI in *C. elegans*, could work as a translational repressor to inhibit P53 expression and

subsequent apoptosis process induced by DNA damaging stimuli [31]. In addition, a pro-apoptotic role of QKI-7 in NIH3T3 cells was reported before and antagonistic effects of QKI-5 and 6 in apoptosis was known to be due to dimerization with QKI-7 [32]. In this regard, although our study favor the model that QKI-5 and QKI-6 protect the cardiac cell from IR induced the apoptosis through FOXO1, we could not exclude the role of QKI7, p53 and some other genes.

Altogether, our data first demonstrated the anti-apoptotic effects of endogenous QKI-5 and -6 in cultured cardiomyocytes and underlying mechanism was at least partially due to repression of FoxO1. Inhibition of FoxO1 by QKI may serve as a homeostatic modulator to negatively regulate the intensity of FOXO1 activation and thus IR induced apoptosis.

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