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YB-1 downregulation attenuates UQCRC1 protein expression level in H9C2 cells and decreases the mitochondrial membrane potential

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Key words: siRNA, Mitochondrial membrane potential, Cardiomyocytes

Abstract: UQCRC1 is one of the 10 mitochondrial complex III subunits, this protein has a role in energy metabolism, myocardial protection, and neurological diseases. The upstream mechanism of the UQCRC1 protective effect on cardiomyocytes is currently unavailable. In order to explore the upstream molecules of UQCRC1 and elucidate the protective mechanism of UQCRC1 on cardiomyocytes in more detail, we focused on the nuclease-sensitive element-binding protein 1 (YB-1). We hypothesized YB-1 acts as an upstream regulatory molecule of UQCRC1. This study found that YB-1 RNAi significantly reduces the expression of the UQCRC1 protein level ($p < 0.05$) and obviously decreases the mitochondrial membrane potential ($p < 0.05$), and that YB-1 interacts with UQCRC1 protein *in vivo*, but YB-1 RNAi has little effect on the UQCRC1 gene transcription.

Introduction

Mitochondrial complex III is part of the mitochondrial electron transport chain, and ubiquinol-cytochrome c reductase core subunit 1 (UQCRC1[GI: 402746975]) is a core subunit of mitochondrial complex III (Chen *et al.*, 2003; Ellinger *et al.*, 2016; Van Ginkel *et al.*, 2015). Studies have found that UQCRC1 is decreased after ischemia-reperfusion (I/R) in isolated rat hearts (Lin *et al.*, 2012). Overexpression of UQCRC1 can enhance the complex III activity (Aguilera-Aguirre *et al.*, 2009; Kriaucionis *et al.*, 2006; Shibanuma *et al.*, 2011). Overexpression of UQCRC1 also can protect H9C2 cells from oxygen-glucose deprivation (OGD) injury by activating the PI3K/Akt signal pathway and the ERK/GSK-3β signal pathway (Baines *et al.*, 2001; Yi *et al.*, 2017).

The oxidative phosphorylation system (OXPHOS) consists of mitochondrial complexes I to IV (Fernandez-Vizarra and Zeviani, 2018), which can produce ATP for energy supply (Tian *et al.*, 2018). UQCRC1, as an OXPHOS protein (Petersson *et al.*, 2014), plays a key role in the assembly of complex III and oxidative phosphorylation (Fernandez-Vizarra and Zeviani, 2018). As a core subunit of mitochondrial complex III, UQCRC1 is assembled with UQCRFS1 to perform the function of complex III (Fernandez-Vizarra and Zeviani, 2018; Iwata *et al.*, 1998). The downstream signaling pathway of myocardial protection by UQCRC1 is well understood, but the upstream regulatory mechanism of UQCRC1 is currently not known.

YB-1 (GI: 92373397), a member of the Y-box family proteins (Lasham *et al.*, 2013), is a DNA-/RNA-binding protein containing a cold shock protein domain (Dong *et al.*, 2009; Minich *et al.*, 1993; Suresh *et al.*, 2018). YB-1 is a transcriptional and translational factor regulating multiple cellular processes such as DNA repairing, cell proliferation, oxidative phosphorylation, and mRNA translation (Kohno *et al.*, 2003; Lasham *et al.*, 2013). YB-1 plays a key role in translational regulation. As a key component of inactive mRNPs (mRNA–protein complexes) (Skabkin *et al.*, 2004), YB-1 can prevent translation initiation and inhibit mRNA degradation (Evdokimova *et al.*, 2001). The regulation of mRNA translation depends on the ratio of YB-1/mRNA (Lyabin *et al.*, 2014).

Previous research has shown that YB-1 is essential for mitochondrial OXPHOS activity and may control OXPHOS protein translation (Matsumoto *et al.*, 2012). Overexpression of UQCRC1 can protect H9C2 cells from OGD injury (Baines *et al.*, 2001; Yi *et al.*, 2017). YB-1 also can protect cardiac myocytes against IR injury (Wang *et al.*, 2019). Thus, both YB-1 and UQCRC1 can protect cardiomyocytes. However, the mechanism for YB-1 regulating UQCRC1 remains unclear. In this study, we predicted that the YB-1 binds to the promoter region of UQCRC1 by bioinformatics and performed chromatin immunoprecipitation (ChIP) to verify two binding sites of UQCRC1. Our study elucidated that YB-1 RNAi induced the

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decrease of UQCRC1 protein expression and mitochondrial membrane potential. We also demonstrate that YB-1 does not regulate UQCRC1 expression as a transcription factor.

Materials and Methods

Cell culture

H9C2 cells line were provided by the Central Laboratory of the Second Affiliated Hospital of the Army Military Medical University. The cells were cultured as previously described (Yi *et al*., 2017).

Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) assays and ChIP-qPCR were performed as described (Wang *et al*., 2017). The antibodies were specific for YB-1 (Abcam, ab76149) or normal rabbit IgG (CST, 2729S). qPCR was performed using SYBR Green reagents with primers listed in Tab. 1. The detection fragments were as follows: Detection fragment 1 (>chr8:117677328-117679427): AGCCGGCCCTGCTGAGGCTACCTGCCTTGCGGGGTACCGCAACCTTCGTCCAGGCCCTCCAGAGCGTGCCGGAGACACAGGTCAGCGTATTGGACAACGGGCTGCGTGTGGCCTCGGAGCAGTCCTCGCATCCT.

Detection fragment 2 (>chr8:117678244-117679431): ATTTCACTTTATTTTCTGCTCTAAATCATATTTCTGGTCCACAAAATGTCAATATACACTAAGTATTGTTTGTCTGATTTCAGATAGTTTTTTTAATGTTTTGAAATAATCACAAATACAGAAAAGACATTTTTTTGTTTAGTTTTTGTTTTCCAAACAAGGAGTCCTATTTCACAAATTACCTCTACACGGTTCC.

The primers YB-1-F/R and UQCRC1-F1/R1 were used for qPCR after YB-1 siRNA, the primers UQCRC1-F2/R2 and UQCRC1-F3/R3 were used for ChIP.

RNA interference

The small interfering RNA (siRNA) sequences that target YB-1 and NC siRNA were synthesized by RiboBio. The sequences for the sense strands of the oligonucleotides were as follows: YB-1 siRNA-1 5'–CCAAGGAAGACGTATTTGT–3', YB-1 siRNA-3 5'–GGAATGACACCAAGGAAGA–3'. Transfection was processed with Lipofectamine 2000 (Invitrogen) in 6-well plates with siRNAs according to transfection reagent protocols. H9C2 cells were transfected with the siRNA oligonucleotides (50–150 nM final). After 48, 60, and 72 h incubation with YB-1 siRNA or NC siRNA, the cells were collected according to experimental time requirements.

TABLE 1

Sequences of primers for quantitative reverse-transcription PCR

Primer name	Primer sequence (5'–3')
YB-1-F	TAAATACGCAGCAGACCG
YB-1-R	CAGCACCCTCCATCACTT
UQCRC1–F1	GGGGCACAAGTGCTATTGC
UQCRC1–R1	GTTGTCCAGCAGGCTAACC
UQCRC1–F2	CCTGCTGAGGCTACCTGC
UQCRC1–R2	AGGATGCGAGGACTGCTC
UQCRC1–F3	TCTCCTGCAGTTGCACAC
UQCRC1–R3	CATCAAGTCCATTGGCAAG

RNA extraction and quantitative reverse-transcription PCR (qPCR)

The total RNA was extracted with RNAiso Plus reagent (Takara, 9108) according to protocol. Then the cDNA was synthesized from total RNA using the PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, RR047A). The cDNA was diluted 10 times as a template. PCR amplification was performed with a total volume of 20 μL. The amplified products were detected using SYBR-green supermix (Takara, RR420L) with ViiA7DX (AB). qPCR was performed as the following conditions: 95°C for 30 s, 40 cycles of 95°C for 3 s, and 60°C for 30 s. The control gene was the actin gene. Primer sequences were listed in Tab. 1. The mean ± SD of the gene expression levels in three independent experiments were reported. The relative values of signals were normalized to the actin controls.

Protein extraction and Western blotting analysis

H9C2 cells were lysed by RIPA buffer, and the samples were centrifuged at 4°C for 15 min at 11000 rpm, the supernatant was collected as the total cell extracts and stored at −80°C. The expression levels of YB-1 and UQCRC1 were examined by Western blotting. H9C2 cells were harvested after 72 h YB-1 RNAi, and the same amounts of protein were analyzed by SDS-PAGE. After transferring the protein to PVDF membranes, the membranes were incubated using the primary antibodies against YB-1 (ABclonal, A7704), UQCRC1 (Proteintech, 21705-1-AP) and GAPDH (Proteintech, 60004-1-Ig). Then the binding of each primary antibody was detected with a secondary antibody at 1:10000 dilution and visualized using the enhanced ECL method. The intensities of antibody complexes were detected with the Image Quant LAS 4000.

Proteins of cytoplasm and nucleus in H9C2 cells were extracted by Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, p0027). Proteins of mitochondrial and cytosolic were extracted by Cell Mitochondria Isolation Kit (Beyotime, C3601). YB-1 and UQCRC1 expression were verified by Western blotting.

Immunofluorescence subcellular localization

H9C2 cells were washed with PBS and fixed with 4% paraformaldehyde for 10 min, and then washed 3 times with PBS for 5 min each time. Then, the cells were perforated with PBST (0.3% Triton X-100) for 10 min and washed 3 times with PBS for 5 min each time. After this step, the cells were blocked with 5% BSA for 1 h at room temperature, incubated with a 1:400 dilution of antibodies (anti-YB-1, anti-Tomm20, and anti-UQCRC1 antibodies) at 4°C overnight, and then washed 3 times with PBS for 5 min each time. The cells were incubated with the secondary antibodies (AlexaFluor® 647 goat anti-rabbit and AlexaFluor® 488 goat anti-mouse for YB-1 and UQCRC1; AlexaFluor® 488 goat anti-mouse and AlexaFluor® 647 goat anti-rabbit for Tomm20 [Abcam, ab56783] and YB-1) (AlexaFluor® 488 goat anti-mouse and AlexaFluor® 647 goat anti-rabbit for Tomm20 and UQCRC1 [Proteintech, 21705-1-AP]) for 1 h at 37°C and washed 5 times with PBS for 5 min each time. The cells were stained with DAPI for 10 min and washed 3 times with PBS for 5 min each time. Finally, laser confocal microscopy was used for photographing and analysis.

Mitochondrial membrane potential assay

The mitochondrial membrane potential was measured using mitochondrial a membrane potential assay kit with JC-1 (Beyotime, C2006). H9C2 cells were cultured in 96-well plates to four groups at an initial density of 5×10^3 cells per well. After 72 h RNA interference, the reaction was carried out at 37°C for 20 min with a JC-1 probe (10 μmol/L reaction concentration). The experiment examined eight different regions, with eight biological replicates per trial and three technical replicates. The analysis was conducted by fluorescence microscopy and ImageJ (NIH).

Co-IP-MS

Firstly, H9C2 cells were washed three times with cold PBS and lysed in RIPA buffer containing the protease inhibitor PMSF (Beyotime, ST506) for 5 min. Then the lysates were centrifuged for 5 min at 12000 rpm at 4°C, and the supernatants with the same amount of total protein were subjected to Co-IP with the UQCRC1 antibody (Proteintech, 21705-1-AP) overnight at 4°C. After electrophoresis, the SDS-PAGE gels were used for Coomassie Brilliant Blue staining, decolorization observation, and mass spectrometry analysis to identify its composition. LC-MS/MS and data analysis were completed by Army Military Medical University Central Laboratory. Finally, the immunoprecipitation protein was verified by Western blotting.

Cell viability assay

The cell viability assay was measured using Enhanced Cell Counting Kit-8 (CCK-8) (Beyotime, C0041). H9C2 cells were cultured in 96-well plates to four groups at an initial density of 5×10^3 cells per well. After 72 h RNA interference, the reaction was incubated at 37°C for 1 h with 10 μL CCK-8 solution, then we measured absorbance at 450 nm. The experiment examined with three biological replicates per trial and six technical replicates.

Statistical analysis

Data are shown as the means ± SEM. The average of two groups was compared using independent *t*-tests. $p < 0.05$ was considered as a significant difference.

Results

YB-1 is not a transcription factor of UQCRC1

To examine whether YB-1 regulates UQCRC1 expression as a transcription factor, we performed ChIP assay. Firstly, we performed agarose gel electrophoresis to detect chromosome breaks. The result showed a good chromosomal breakage (Fig. 1A); it could be used for further experiments. Then we measured the binding of YB-1 to the UQCRC1 promoter region in different fragments by qPCR. The results showed that YB-1 did not bind to the promoter region of UQCRC1 (Fig. 1B). Therefore, YB-1 did not regulate UQCRC1 as a transcription factor.

YB-1 down-regulated UQCRC1 protein expression but did not decrease mRNA expression after YB-1 RNAi

To further verify the relationship between YB-1 and UQCRC1 interactions, we performed qPCR and Western blotting after YB-1 RNAi. Transfection of three YB-1 RNAi fragments revealed that the expression of YB-1 mRNA was silenced significantly by the interference fragments 1 and 3. The best interference concentration and interference time were explored with these two fragments. The results showed that the interference effect of 150 nM concentration was very similar to 100 nM, so the interference of 100 nM was the best interference concentration (Figs. 1C, 1D). After YB-1 RNAi 48, 60, and 72 h, YB-1 mRNA can be silenced by fragment 1 about 75%, 60%, 65%, respectively. And YB-1 mRNA can be silenced by fragment 3 about 50%, 65%, 55%, respectively (Figs. 1C, 1D). The expression of UQCRC1 mRNA was measured by qPCR. The UQCRC1 gene did not show the same decreasing trend with the decrease of the YB-1 gene level (Figs. 1E, 1F).

We examined the expression of YB-1 protein after 72 h in different interference concentrations by Western blotting. The results showed that 50 nM fragments 1 and 3 silenced about 60% and 50%, 100 nM fragments 1 and 3 silenced about 50% and 45%, 150 nM fragments 1 and 3 silenced about 65% and 50% (Figs. 2A–2C), which were consistent with the downward trend of the gene level (Figs. 1C, 1D). In the meantime, the expression of the UQCRC1 protein was measured after 72 h interference of YB-1. The expression of the UQCRC1 protein was consistent with the YB-1 protein expression after interference (Figs. 2B–2D). Protein expression of UQCRC1 decreased with increasing concentration of YB-1 RNAi. The interference experiment indicates that YB-1 can regulate UQCRC1 protein expression.

Decrease of YB-1 and UQCRC1 after YB-1 RNAi were mainly in cytoplasm

In addition, we examined the subcellular localization of YB-1, Tomm20, and UQCRC1 in H9C2 cells by immunofluorescence. At the same exposure intensity, YB-1 and UQCRC1 were localized in the cytoplasm of H9C2 cells (Fig. 3A). After YB-1 RNAi (150 nM), the expression of YB-1 in cytoplasm significantly decreased (Fig. 3A) as compared to the NC group, and the expression of UQCRC1 also decreased (Figs. 3A, 3B). After YB-1 RNAi, the expression of Tomm20 also decreased (Figs. 3B, 3C). Statistical analysis of fluorescence intensity was performed by ImageJ, and the results were significantly different (Fig. 3D). In H9C2 cells, YB-1, UQCRC1, and Tomm20 were localized in the cytoplasm (Fig. 3E). Western blotting results showed YB-1 and UQCRC1 were mainly in the cytoplasm, and YB-1 was slightly expressed in the nucleus (Fig. 3F). The results for mitochondrial protein showed that UQCRC1 was expressed in mitochondria and was not detected in mitochondria-free cytoplasm (Fig. 3G). YB-1 was expressed in both mitochondria and mitochondria-free cytoplasm and was abundantly expressed in the cytoplasm (Fig. 3G). Western blotting detections of protein distribution in the cells were consistent with the immunofluorescence localization. These experiments showed that YB-1 acts as a regulatory molecule of UQCRC1, and YB-1 may affect mitochondrial outer membrane protein Tomm20 by regulating mitochondrial protein UQCRC1.

YB-1 RNAi decreased the mitochondrial membrane potential

The decrease of mitochondrial membrane potential can be easily detected by the transition of red fluorescence of JC-1 to green fluorescence. After YB-1 RNAi (150 nM), the membrane potential of mitochondria was significantly lower

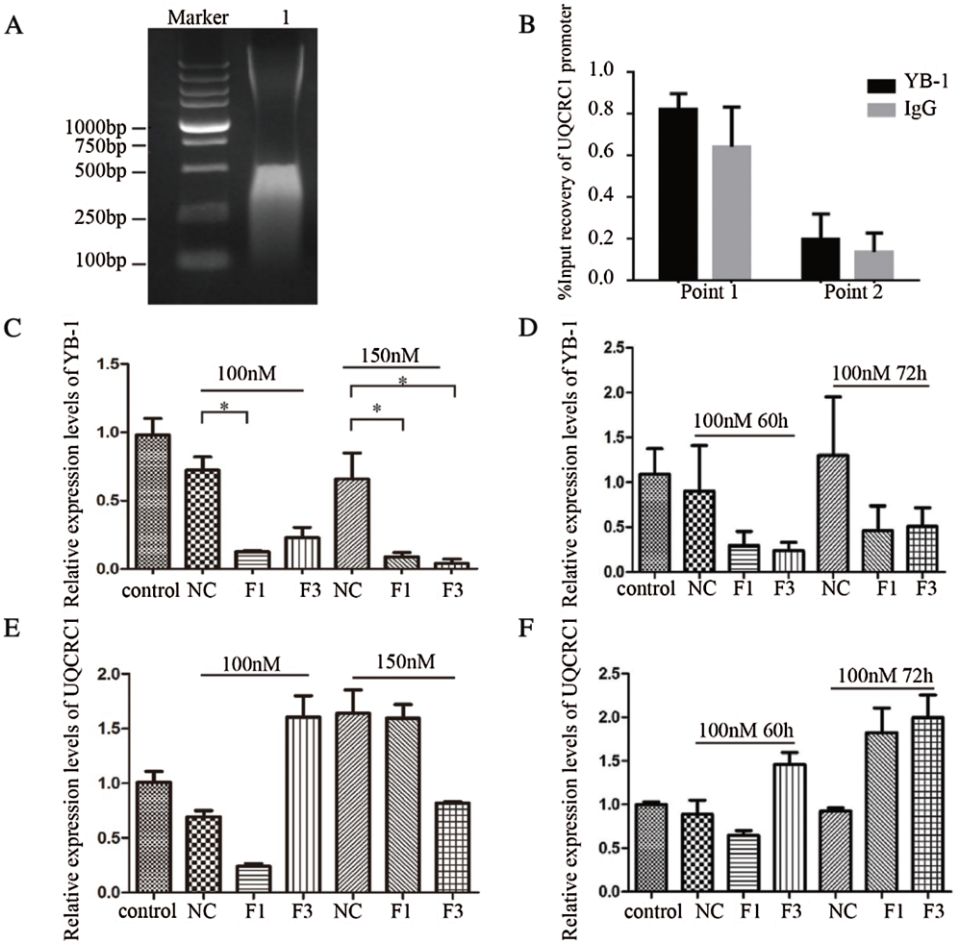

FIGURE 1. The expression of the YB-1 gene and the UQCRC1 gene in H9C2 by qPCR.
(A) Chromosome fragmentation electropherogram. Lane 1. Chromosomal breaks and purified DNA. (B) ChIP verification YB-1 combined with the UQCRC1 promoter region. Point 1and point 2 are two different segments in the promoter region, respectively. (C) The expression of the YB-1 gene after YB-1 RNAi 48 h. The 100 nM and 150 nM are the concentration of YB-1 RNAi fragments. (D) The expression of the YB-1 gene after YB-1 RNAi (100 nM) 60 h and 72 h. (E) The expression of the UQCRC1 gene after YB-1 RNAi 48 h. (F) The expression of the UQCRC1 gene after YB-1 RNAi (100 nM) 60 h and 72 h. F1, YB-1 RNAi fragment 1. F3, YB-1 RNAi fragment 3. $*p < 0.05$ compared with the NC group. The experiments consisted of nine technical replicates and three biological replicates.

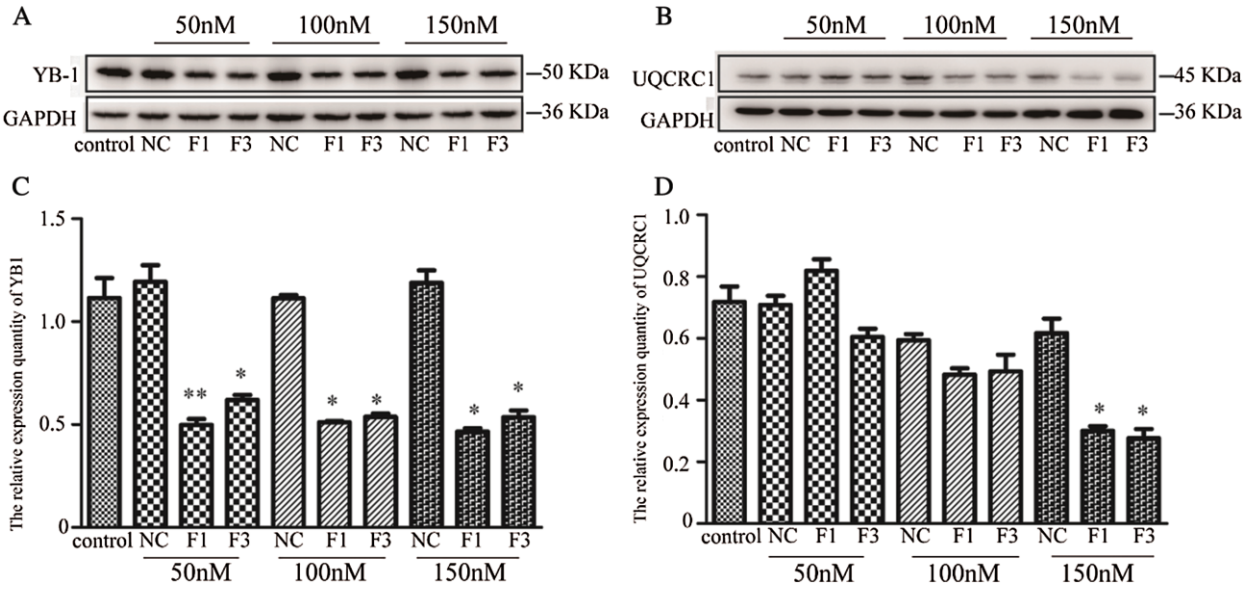

FIGURE 2. The expression of the YB-1 protein and the UQCRC1 protein in H9C2 after YB-1 RNAi by Western blotting.
(A) The expression of the YB-1 protein after YB-1 RNAi. (B) The expression of the UQCRC1 protein after YB-1 RNAi. (C) The relative expression of the YB-1 protein after YB-1 RNAi. (D) The relative expression of the UQCRC1 protein after YB-1 RNAi. NC, NC group. F1, YB-1 RNAi fragment 1. F3, YB-1 RNAi fragment 3. $*p < 0.05$ compared with the NC group. The experiments consisted of nine technical replicates and three biological replicates.

than that of the control and the NC RNAi group (Fig. 4A). Green fluorescence of the YB-1 RNAi group was stronger than the control and NC RNAi group based on fluorescence intensity statistics of image J (Fig. 4B), and CCCP was a positive control. Since YB-1 RNAi reduced the expression of UQCRC1, the core protein of mitochondrial complex III, which in turn affected the membrane potential of mitochondria.

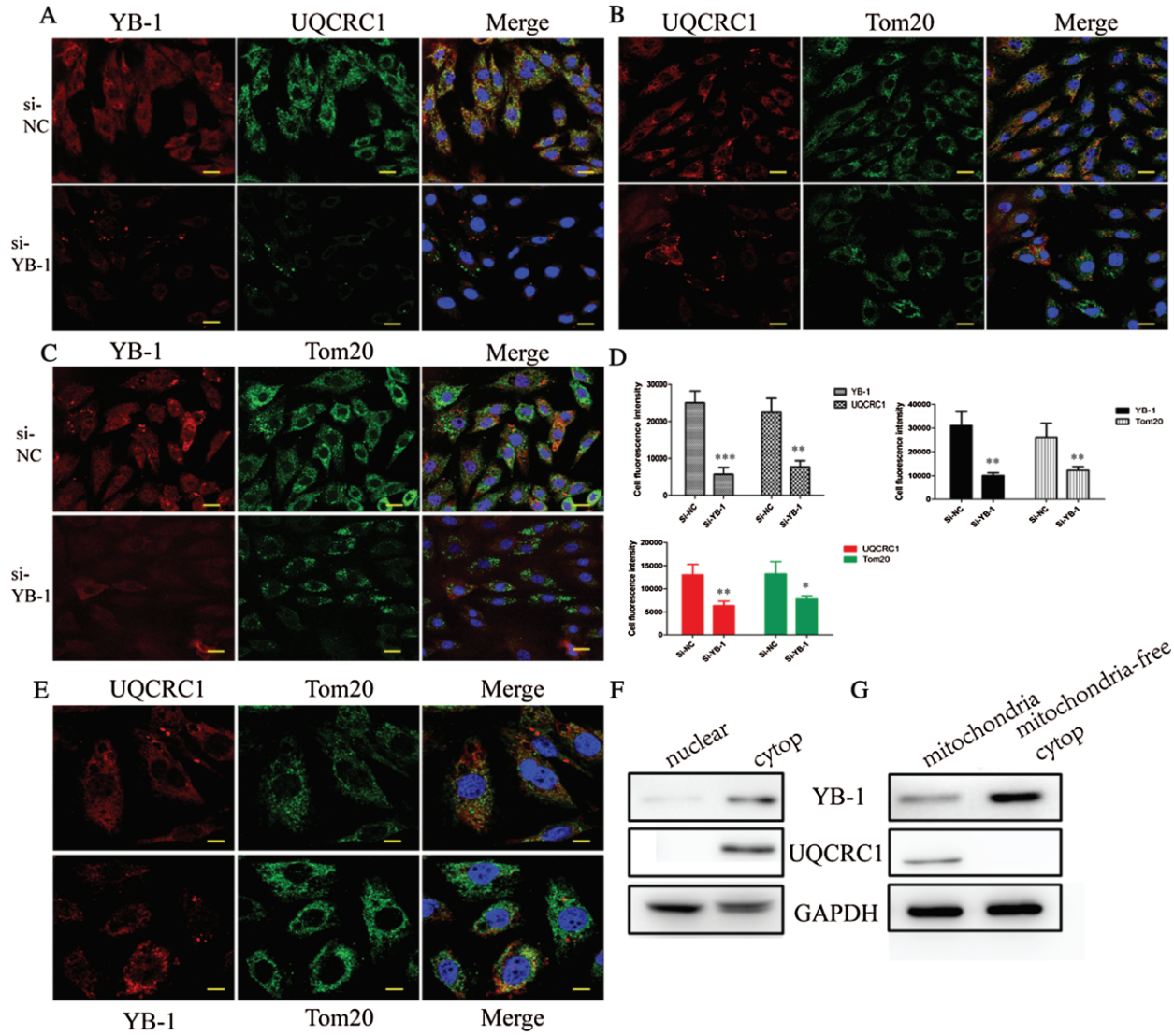

FIGURE 3. Immunofluorescence co-localization for YB-1, Tomm20, and UQCRC1 in H9C2.
(A) The co-localization of YB-1 and UQCRC1. (B) The co-localization of Tomm20 and UQCRC1. (C) The co-localization of YB-1 and Tomm20. The anti-YB-1 antibody was visualized with AlexaFluor® 647 secondary antibody (red) in A and C. Anti-UQCRC1 antibody was visualized with AlexaFluor® 488 secondary antibody (green) in A, and AlexaFluor® 647 secondary antibody (red) in B. Si-NC was NC RNAi group, si-YB-1 was YB-1 RNAi group. (D) ImageJ performs quantitative analysis of fluorescence intensity 30 cells were randomly selected from each group for counting. Scale bar is 30 μm. (E) The co-localization of Tomm20 and UQCRC1 and the co-localization of YB-1 and Tomm20 with higher magnification without RNAi. (F) Protein expression detection of YB-1 and UQCRC1 in the cytoplasm and nucleus by Western blotting. (G) Protein expression detection of YB-1 and UQCRC1 in mitochondria and mitochondria-free cytoplasm. Scale bar is 15 μm. The experiments consisted of 9 technical replicates and 3 biological replicates.

YB-1 interacts with the UQCRC1 protein

To verify the interaction between YB-1 and UQCRC1, we performed Co-IP-MS. Mass spectrometry results of co-immunoprecipitation (Tab. 2) revealed that YB-1 interacts with UQCRC1. The Western blotting results confirmed the interaction of UQCRC1 with YB-1 protein (Fig. 4C). Therefore, this result suggested that YB-1 protein bound to UQCRC1 protein *in vivo*. The interaction of these two proteins was not known before.

The decrease of YB-1 attenuates H9C2 cellular activity

After YB-1 RNAi of the concentration 150 nM, the cell viability was detected by CCK-8 kit. The cell viability was significantly reduced after si-YB-1 compared with the si-NC group (Fig. 4D). The interference of YB-1 affected the expression of UQCRC1 protein and mitochondrial membrane potential and further affected cell viability by affecting mitochondrial activity.

Discussion

This study demonstrated for the first time that YB-1 RNAi attenuated the UQCRC1 protein expression level in H9C2 cells and decreased the mitochondrial membrane potential. Consistent with a previous study (De Souza-Pinto *et al.*, 2009), the current study confirmed that YB-1 protein was mainly localized to the cytoplasm in H9C2 cells. Upon YB-1 RNAi, the cytoplasmic levels of YB-1 and UQCRC1 were significantly reduced. And YB-1 RNAi (150 nM) decreased the protein expression of UQCRC1

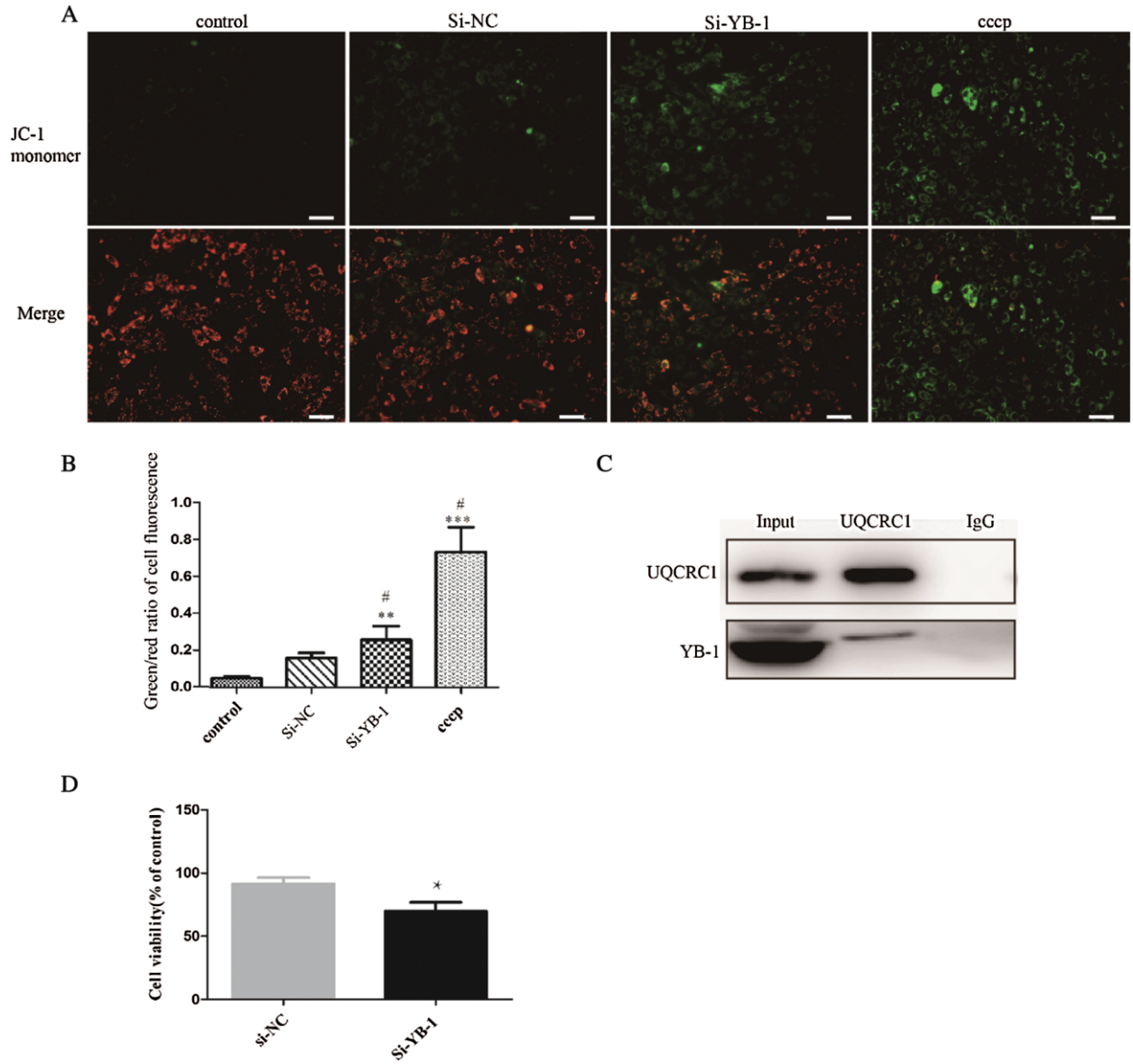

FIGURE 4. Detection of mitochondrial membrane potential and cell viability.
(A) JC-1 monomer detected by green fluorescence, representing a decrease in mitochondrial membrane potential. Merge is the overlay of red fluorescence and green fluorescence of mitochondrial membrane potential. (B) The statistics of the ratio of green fluorescence to red fluorescence intensity by image J. (C) Western blotting detection of co-immunoprecipitation. H9C2 cells were immunoprecipitated with anti-UQCRC1 antibody, with IgG as a control. And the immunoprecipitated complex was analyzed using Western blotting. (D) CCK-8 kit detected the cell activity after si-YB-1. $^{*}p < 0.05$ compared with the normal group. $^{\#}p < 0.05$ compared with the NC RNAi group. Scale bar is 400 μm.

TABLE 2

List of UQCRC1 Co-IP binding proteins identified by mass spectrometry

Protein name and symbol	MW (KDa)
Nuclease-sensitive element-binding protein 1 OS = *Rattus norvegicus* GN = Ybx1 PE = 1 SV = 3 − [YBOX1_RAT]	35.7
40S ribosomal protein S6 OS = *Rattus norvegicus* GN = Rps6 PE = 1 SV = 1 − [RS6_RAT]	28.7
60S ribosomal protein L7 OS = *Rattus norvegicus* GN = Rpl7 PE = 1 SV = 2 − [RL7_RAT]	30.3
78 kDa glucose-regulated protein OS = *Rattus norvegicus* GN = Hspa5 PE = 1 SV = 1 − [GRP78_RAT]	72.3
Actin, cytoplasmic 1 OS = *Rattus norvegicus* GN = Actb PE = 1 SV = 1 − [ACTB_RAT]	41.7
STE20-like serine/threonine-protein kinase OS = *Rattus norvegicus* GN = Slk PE = 1 SV = 1 − [SLK_RAT]	137.8
Myosin-9 OS = *Rattus norvegicus* GN = Myh9 PE = 1 SV = 3 − [MYH9_RAT]	226.2
Keratin, type I cytoskeletal 10 OS = *Rattus norvegicus* GN = Krt10 PE = 2 SV = 1 − [K1C10_RAT]	56.5

significantly, but the higher concentration actually weakened, probably because of the off-target effect. Our data suggested that YB-1 interacted with UQCRC1 and regulated UQCRC1 protein expression, but it did not decrease UQCRC1 transcription.

In addition, the regulation of translational levels varies under different conditions. Many mRNA-binding proteins were involved in translational regulation (Skabkina *et al.*, 2003). YB-1, an mRNA-binding protein, could regulate the gene expression in both transcriptional and translational modes (Kohno *et al.*, 2003; Matsumoto and Wolffe, 1998). In particular, YB-1 could keep mRNA in a silent state by binding to mRNA to form a translationally inactive mRNPs (Somasekharan *et al.*, 2015; Wang *et al.*, 2017). The inactive mRNPs and translational initiation factors, such as eIF4E, formed the translational p-bodies (PBs), which were stored in the cytoplasm (Anderson and Kedersha, 2008; Somasekharan *et al.*, 2015). And the functions of YB-1 as either an inhibitor or activator of translation depended on the molar ratio of YB-1 to its target mRNA (Evdokimova and Ovchinnikov, 1999; Matsumoto *et al.*, 2012).

UQCRC1 is one of the YB-1 mRNA-binding targets. Our study indicated that UQCRC1 mRNA expression did not decrease after YB-1 RNAi. Thus YB-1 RNAi did not affect UQCRC1 mRNA expression. YB-1 was correlated with the protein regulation of UQCRC1. When interfering YB-1, the ratio of YB-1/mRNA decreases (Evdokimova *et al.*, 1998), and UQCRC1 mRNA translation is inhibited, which leads to a decrease in the expression of UQCRC1 protein. The downregulation of UQCRC1 protein affects the activity of mitochondrial complex III (Deng *et al.*, 1998; Fernandez-Vizarra and Zeviani, 2018; Glaser and Dessi, 1999). Complex III is a key component of electron transfer in mitochondria. When the activity of complex III decreases, it affects the proton transfer in the respiratory chain and reduces the mitochondrial membrane potential (Maianski *et al.*, 2004; Van Raam *et al.*, 2008). Therefore, YB-1 may regulate the translation of UQCRC1. The depletion of YB-1 leads to the downregulation of UQCRC1 protein expression and decrease of mitochondrial membrane potential through induction of protein translation from mRNPs release (Evdokimova and Ovchinnikov, 1999; Skabkin *et al.*, 2004).

Mitochondrial oxidative phosphorylation is responsible for the production of ATP in cells (Stock *et al.*, 1999; Wittig *et al.*, 2006). Mitochondrial complex III is the third of five OXPHOS complexes (Fiorillo *et al.*, 2016; Lei *et al.*, 2017), the decrease in complex III activity can be caused by the mutation of the complex III subunit gene (Fernandez-Vizarra and Zeviani, 2018). The nuclear-encoding gene UQCRC1 is a core protein of complex III. Therefore, the changes in UQCRC1 protein expression can lead to a change in the activity of complex III (Kriaucionis *et al.*, 2006). Previous studies have studied the protective effect of UQCRC1 on myocardial OGD injury and the downstream signaling pathway (Long *et al.*, 2017; Yi *et al.*, 2017). The transport of UQCRC1 from the nucleus to mitochondria might be closely related to YB-1. Identification of YB-1 as a regulatory protein of UQCRC1 lays a foundation for further study on the myocardial protection signaling pathways.

Conclusion

In conclusion, through a series of biochemical studies, we investigated the relationship between YB-1 and UQCRC1 in H9C2 cells. This work demonstrated that YB-1 RNAi attenuated UQCRC1 protein expression in H9C2 cells and decreased the mitochondrial membrane potential. YB-1 interacted with UQCRC1 protein in H9C2 cell lines.

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Conflicts of Interest: No competing financial interests exist.

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