Biochemical and Biophysical Research Communications 474 (2016) 168–174

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Up-regulation of miRNA-221 inhibits hypoxia/reoxygenation-induced autophagy through the DDIT4/mTORC1 and Tp53inp1/p62 pathways

Qiying Chen [a], Yue Zhou [b, c], A. Mark Richards [b, c, d, e], Peipei Wang [b, c, *]

[a] Huashan Hospital, Fudan University, Shanghai, China
[b] Cardiovascular Research Institute, National University Health System, Singapore
[c] Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[d] Cardiac Department, National University Health System, Singapore
[e] Christchurch Heart Institute, Department of Medicine, University of Otago-Christchurch, Christchurch, New Zealand

ARTICLE INFO

Article history:
Received 10 April 2016
Accepted 14 April 2016
Available online 20 April 2016

Keywords:
miRNA-221
Ddit4
Tp53inp1
Autophagy
Ischemia/reperfusion

ABSTRACT

Timely reperfusion in acute myocardial infarction has improved clinical outcomes but the benefits are partially offset by ischemia-reperfusion injury (I/R). MiRNA regulates mRNA of multiple effectors within injury and survival cell signaling pathways. We have previously reported the protective effects of miRNA-221 in I/R injury. The purpose of this study was to explore the mechanisms underlying cardioprotection of miR-221. Myoblast H9c2 and neonatal rat ventricular myocytes (NRVM) were subjected to 0.2% O_2 hypoxia followed by 2 h of re-oxygenation (H/R). In gain-and-loss function studies through transfections of miR-221 mimic (miR-221) and inhibitor (miR-221-i), the protective effects of miR-221 were confirmed as assessed by increased cell metabolic activity (WST-1) and decreased LDH release. Autophagy was assessed by GFP-LC3 labeling of autophagosome formation, LC3 and p62 measurements. Co-immunoprecipitation and specific gene cloning and function were used to identify the pathways underpinning miR-221 effects. MiR-221 significantly reduced H/R injury in association with inhibition of autophagy. Underlying mechanisms include (1) down-regulation of Ddit4 (disinhibiting the mTORC1/p-4EBP1 pathway) which inhibits autophagosome formation (2) down-regulation of Tp53inp1 (with reduced Tp53inp1/p62 complex formation) which inhibits autophagosome degradation. In conclusion, miRNA-221 exerts cytoprotective effects in hypoxia-reoxygenation injury in association with alterations in autophagic cell injury. Mir-221 may constitute is a novel therapeutic target in the treatment of cardiac I/R injury.

1. Introduction

In acute myocardial infarction (MI), restoration of coronary blood flow (reperfusion) offers major clinical benefits but gains are partially offset by ischemia/reperfusion injury (I/R) which may increase risks of heart failure (HF) and mortality. Thus the prevention or amelioration of I/R injury constitutes a major unmet need. Reperfusion-induced oxidative stress, intracellular Ca^{2+} overload, mitochondrial dysfunction and inflammation may trigger cardiac cell autophagy, apoptosis and ultimately cell death [1–3]. Therefore, regulation of I/R-induced autophagy and apoptosis may offer a path to minimize reperfusion injury and optimize the benefits.

During myocardial ischemia, ATP and nutrient depletion activates autophagy [3]. This response may beneficially replenish metabolic substrates and remove damaged organelles [4]. However, during reperfusion up-regulated autophagy may accelerate detrimental effects [5]. Specific molecular mechanisms underpin the initiation, elongation and degradation of autophagy. Deactivation of mammalian target of rapamycin complex 1 (mTORC1), an important nutrient and stress sensor, leads to the initiation of autophagy [6]. Multiple genes regulate mTORC1 activity, including DNA-damage inducible transcript 4 (Ddit4) [7]. Autophagosome formation involves multiple signaling regulators, most importantly LC3 and Beclin-1 [8,9]. Within autophagosomes engulfed proteins or cytosolic components are degraded by the ubiquitin-proteasome

* Corresponding author. Cardiovascular Research Institute, Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, Centre for Translational Medicine, 14 Medical Drive, 117599, Singapore.
E-mail address: mdcwp@nus.edu.sg (P. Wang).

http://dx.doi.org/10.1016/j.bbrc.2016.04.090
0006-291X/

or autophagy-lysosomal system [10]. P62/SQSMT1 (p62) plays a central role in this process. As an autophagy adapter, multiple binding sites on p62 interact with ubiquitinated-proteins [11], and many cell signaling pathways including LC3, TRAF6, PKC and NF-κB [10]. It may interact with tumor protein 53-induced nuclear protein 1 (Tp53inp1) and Tp53inp2 to regulate protein degradation be expressed across different species [12].

The seed region of miRNA nucleotides binds to complementary mRNA sequences in the 3′-untranslated region (3′-UTR) assembling miRNA to RNA-induced silencing complexes (RISCs) through which mRNAs expression are negatively regulated by translational repression or post-translational target mRNA degradation [13]. Up to 90% of mammalian genes are believed to be regulated by miRNAs [14]. Each miRNA potentially regulates multiple mRNAs to influence the expression of entire networks of genes. Such powerful integrated effects from miRNAs may influence I/R injury by regulating autophagy and/or apoptosis [15]. Taking a combined approaches of miRNA array to select miRNAs up-regulated by cardioprotective agents followed by in vitro functional screening, we previously demonstrated up-regulation of miRNA-221 is associated with a protective effect against I/R [16]. In addition, others have reported that miRNA-221 targeting p27 plays a role in HF through regulation of autophagy [17]. Thus the purpose of this study was to investigate mechanisms through which miRNA-221 regulates I/R-induced-autophagy. We undertook in vitro assessments of the effects of miR-221 upon autophagy. Our data demonstrate that up-regulation of miRNA-221 inhibits I/R-induced autophagy through regulating the Ddit4/mTORC1 and Tp53inp1/p62. MiRNA-221 warrants further in vivo investigation as a potential target for therapeutic interventions to ameliorate I/R injury.

2. Material and methods

2.1. Neonatal rat ventricular myocyte (NRVM) isolation and culture

NRVM were isolated from 1 to 3 day old rats [16]. Minced cardiac ventricles were digested in ~0.4 mg/ml collagenase type II (LS004177, Worthington Biochemical) followed by Percoll (17-0891-01, GE Healthcare) density gradient centrifugation. Cardiomyocytes were seeded for overnight attachment; the culture was maintained in the media containing 50 μM cytosine arabinoside-C (C3350000, Sigma–Aldrich).

2.2. Transfection of miRNA mimics or inhibitors into H9c2 and NRVM

Following the manufacturers' protocol, 25 nM miRNA-221 mimic (miR-221) or inhibitor (miR-221-i) were incubated with lipofectamine 2000 (11668-019, Thermo-Fisher Scientific) at a ratio of 20 pmol: 1 μl, then incubated with cells for 24hr. Mimic control (MC) and inhibitor control (IC) were transfected under the same conditions.

2.3. Cell injury determination of H9c2 and NRVM

H9c2 and NRVM were subjected to a hypoxia/reoxygenation protocol (H/R). (Details in the supplement). At the end, culture medium was collected for the measurement of LDH release, an indicator of cell death (TOX7, Sigma–Aldrich). Cells were lysed for the assay of WST-1 (ab155902, Abcam), a measure of cell mitochondrial dehydrogenase activity as an indicator of cell number and metabolic activity.

2.4. Autophagosome formation assessed by GFP-LC3

H9c2 cells grown on cover slides were transfected with plasmid pEGFP-LC3 (a gift from Dr. Jianghua Li) for 24 h [18]. Cells were treated with (i) 1 μM Rapamycin (an inhibitor of mTORC1 as a positive control for autophagy), (ii) miRNA-221, or (iii) MC for another 24 h and then subjected to H/R. Cells were fixed with 10% formalin and the nuclei stained with 4′-6-diamidino-2-phenylindole (DAPI; blue stain; D1306, Invitrogen). GFP-LC3 fluorescence was observed under a confocal fluorescence microscope (Olympus, Fluoview FV1000). Cells containing five or more GFP-LC3 puncta were defined as autophagy positive cells. 100 or more cells were counted in each sample to count percentage of GFP-positive cells [19].

2.5. Western blot analysis

Cells were lysed and samples of 25 μg protein were loaded onto 10% SDS-PAGE gels for electrophoresis then transferred to PVDF membranes. Membranes were probed with antibodies recognizing LC-3, p62, Beclin-1, phosphorylated (Ser2448) and total mTOR (p-/t-mTOR), phosphorylated and total 4EBP1 (p-/t-4EBP1), phosphorylated and total p70s6k (p-/t-p70s6k), (Cell Signaling Technology). β-actin (Santa Cruz) was used as a loading control.

2.6. Cloning cDNA of genes of interest and recovery study

Sequences of genes of interest were acquired by reverse transcription-PCR and then cloned into the pCMV-Myc (631604, Clontech Laboratories) at restriction enzyme sites EcoRI and NotI, or the pcDNA3.1-Flag (V790-20, Invitrogen, Life Technologies) at restriction enzyme site: EcoRI and NotI. All constructs were confirmed by DNA sequencing (Axil Scientific Pte Ltd, Singapore). The expression of specific proteins was confirmed by WB. For the recovery study, plasmid of the gene of interest and miR-221 mimic were co-transfected into H9c2. LDH release was measured following H/R [20].

2.7. Co-immunoprecipitation to assess complex formation of p62 or Beclin-1 with Tp53inp1

To determine LC3/p62, Tp53inp1/p62 and Tp53inp1/Beclin-1 interaction, pCMV-Myc-LC3, pCMV-Myc-Tp53inp1, pcDNA3.1-Flag-p62 and pcDNA3.1-Flag-Beclin1 were cloned and transfected into H9c2. After transfection for 48 h, cells were lysed. The supernatants were incubated with protein G beads at 4 °C for 1 h. Proteins were lysed from beads then LC3 and Tp53inp1 were pulled down with an anti-Myc antibody. IgG was used as pull-down control. Immunoprecipitates of LC3 and Tp53inp1 were co-incubated with anti-p62 or anti-Beclin1.

2.8. Statistics

All analyses were performed using Graph Pad Prism (San Diego, CA, USA). All values are presented as mean ± SEM. A p value of less than 0.05 was considered statistically significant. Data were compared for differences by one-way ANOVA followed by Bonferroni post-hoc analysis or the unpaired two tail t test as appropriate. Comparisons among multiple groups were analyzed by two-way ANOVA followed by Bonferroni post-hoc analysis.

3. Results

3.1. Protective effects of miR-221 in H9c2 and NRVM H/R-injury model

The protective effects of miR-221 against H/R were confirmed as indicated by increases of cell number and viability (WST-1) and decrease of LDH release ($p < 0.001$ vs. MC) in H9c2 and NRVM. Conversely, miR-221-i adversely affected WST and LDH release (Fig. 1). RISC-loaded miR-221 after mimic and inhibitor transfection was increased for ~100 folds vs. MC and decreased to <10% vs. IC respectively (S.1). The expressions of other miRNAs were not affected as indicated by the measurement of miR-27a and miR-125b (a random selection) in the same Ago-2 IP pull-down.

3.2. H/R-induced autophagosome formation was attenuated by miR-221

Punctate structures, primarily represent autophagosomes, were assessed by exogenously introduced GFP-LC3. Analysis of DAPI and GFP images show that miR-221 significantly reduced H/R-induced autophagosome formation (Fig. 2 a). MC had no such effect and Rap treatment was used as a positive control. The percentage of GFP-LC3 puncta positive cells was reduced from about 50% to 20% (MC vs. miR-221, $p < 0.001$) (Fig. 2 b). The inhibition of autophagy by miR-221 was further supported by the facts that the LC3 II/I ratio was markedly reduced and p62 was up-regulated (Fig. 2 c). MiR-221-i shifted the expression of LC3 II/I and p62 in the opposite direction to that induced by miR-221 (Fig. 2 d). Beclin1 expression was unchanged. Autophagy flux was examined as well. LC3-II and LC3-II/I ratios were significantly increased with Bafilomycin (Baf) treatment. Interestingly, miR-221 increased both LC3-I and LC3-II significantly in comparison with MC with and without Baf. Since the increase of LC-I was much more than LC3-II, LC3 II/I ratio was reduced (S. 2).

3.3. Validation of autophagy-regulated miR-221 targets by luciferase reporter assays and assessment of autophagy flux

We have previously reported that predicted miR-221 targets Ddit4, Tp53inp1 and Tp53inp2 were up-regulated following H/R and reduced by miR-221 [16]. To further validate whether miR-221 directly recognize the 3′UTR of these targets, we constructed luciferase reporter. The predicted binding site(s) of Ddit4, Tp53inp1 and Tp53inp2 are shown in S. 3a. Luciferase reporter assays confirmed miR-221 targeted Ddit4 but not Tp53inp2 (S. 3b). There was no Tp53inp1 luciferase result acquired due to incomplete rat Tp53inp1 3′-UTR sequence information (http://www.ncbi.nlm.nih.gov/nuccore/NM_181084.2). However, Tp53inp1 was considered as a target of miR-221 since it was decreased by miR-221 and increased by MiR-221-i (S. 3 c and d).

3.4. miR-221 inhibited autophagy through targeting the Ddit4/mTORC1 pathway

To demonstrate whether miR-221 inhibits autophagy through Ddit4/mTORC1 pathway, the activation (phosphorylation) of mTOR and downstreams of mTORC1 were assessed in H9c2. MiR-221 increased the ratios of p-/t-mTOR and p-/t-4EBP1 but did not affect p-/t-p70s6K. To further investigate the effects of miR-221 on the mTORC1 pathway, cells undergoing H/R were concurrently treated with Rapamycin (Rap, Rap + MC, Rap + miR-221). MiR-221 reversed Rapamycin-induced inhibition of the mTORC1 pathway, as shown by increased p-/t-mTOR, p-/t-4EBP1 and even p-/t-p70s6K compared to Rap-MC (Fig. 3 a). MiR-221 also reduced cell injury caused by additional Rapamycin (Fig. 3 b ***$p < 0.001$ vs. Rap + MC, ###$p < 0.001$ vs. Rap). MiR-221-i induced opposite effects of reductions in p-/t-mTOR and p-/t-4EBP1 (S. 4).

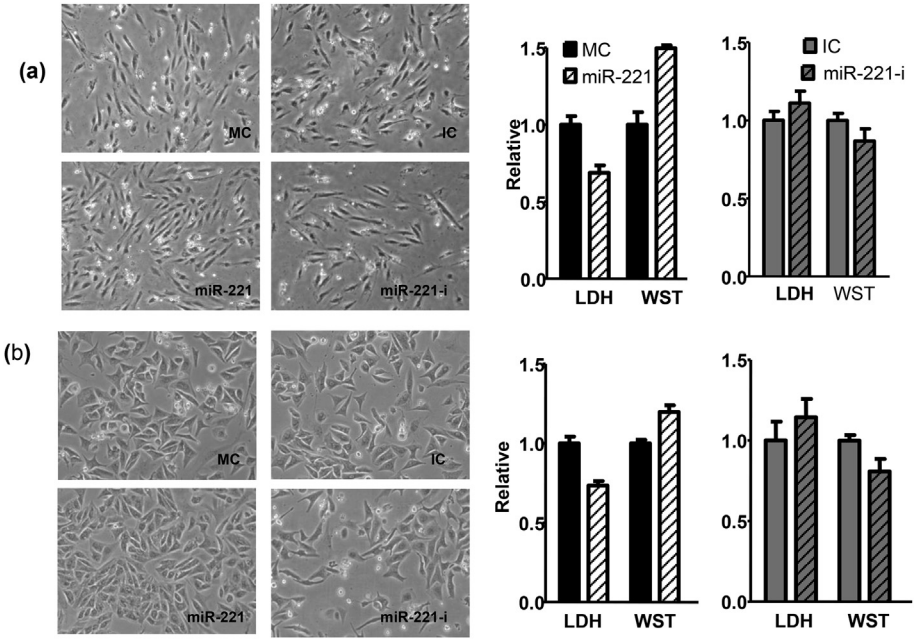

Fig. 1. Protective effects of miR-221 upregulation in H/R model of H9c2 and NRVM. 25 nM miR-221 mimic or mimic control (miR-221 or MC), miR-221 inhibitor or inhibitor control (miR-221-i or IC) were transfected to H9c2 or NRVM. Cells were stressed by 15 h or 6 h of hypoxia (0.2% oxygen) respectively followed by 2 h of re-oxygenation (H/R). Cell morphology, WST-1 activity, LDH release following miR-221 mimic or inhibitor transfection in H9c2 (a) and in NRVM (b). **$p < 0.01$ and ***$p < 0.001$ vs. MC by two-tail unpaired t-test. Each experiment was conducted 3 times in triplicate.

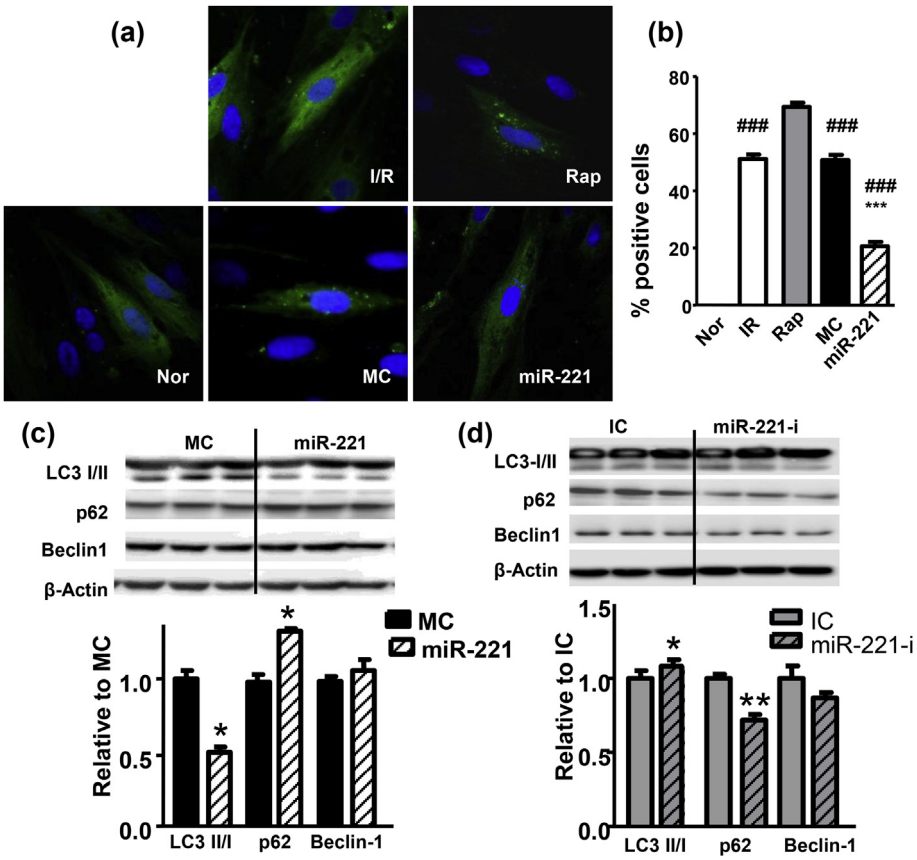

Fig. 2. miR-221 mimic inhibited H/R-induced autophagosome formation. (a) Confocal microscopy showing merged Dapi and GFP image. (b) Calculations of autophagosome positive cell. Total of 100 cells were counted for each group. Rapamycin (Rap) treated as positive control. (c) Expression of LC3, p62 and Beclin1 were detected by Western blot after miR-221 mimics and mimic control transfection and (d) after miR-221 inhibitor and inhibitor control transfection. ###$p < 0.001$ vs. Rap ***$p < 0.001$ vs. MC by two-tail unpaired t-test. Each experiment was conducted 3 times in triplicate.

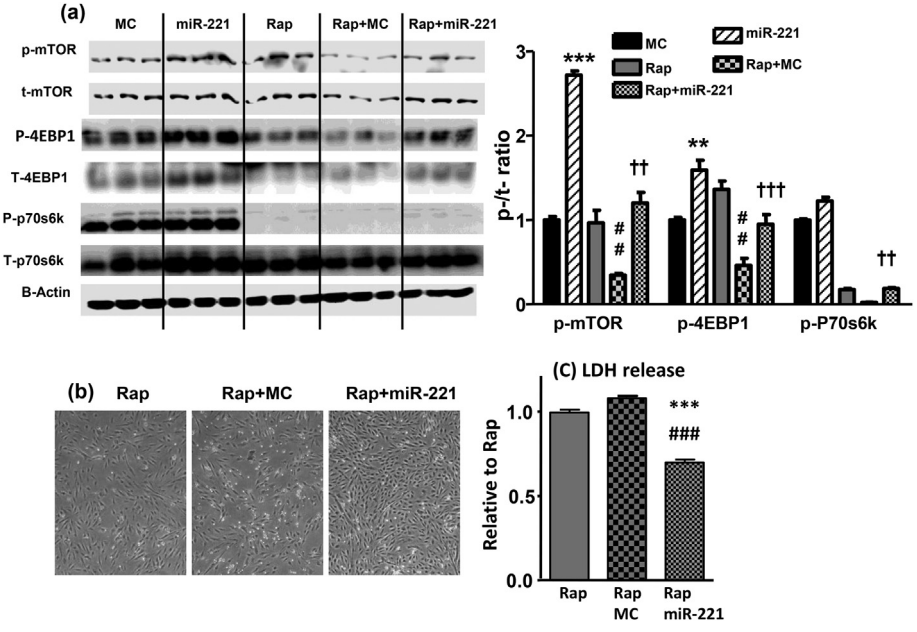

Fig. 3. miR-221 mimic reversed H/R-induced as well as Rapamycin-induced mTORC1 pathway inhibition. (a) Effects of miR-221 mimic on the pathway of p-/t-mTOR, p-/t-4EBP4, p-/t-p70s6k measured by Western blot. (b) miR-221 mimic reduced rapamycin-induced injury (left: cell morphology; right: LDH release). Rap = rapamycin. ***$p < 0.001$ vs. MC; ###$p < 0.001$ vs. Rap; †††$p < 0.001$ vs. Rap-MC by two-tail unpaired t-test. N = 3 in triplicate.

3.5. miR-221 reduced autophagy in association with inhibition of Tp53inp1/p62 complex formation

pCMV-Myc-LC3, pCMV-Myc-Tp53inp1 and pcDNA3.1-Flag-p62 and pcDNA3.1-Flag-Beclin-1 plasmids were cloned (S. 5a). pCMV-Myc-LC3 or pCMV-Myc-Tp53inp1 were transfected into H9c2 for co-IP studies. WB indicated that LC3 and Tp53inp1 were successfully pulled down by anti-Myc antibody (Fig. 4a upper). Probing with p62 antibody indicated the formations of p62-LC3 complex and p62-Tp53inp1 complex (Fig. 4a lower). In another Tp53inp1 anti-Myc pull-down, Beclin-1 was not detectable (Fig. 4b). This indicated that Tp53inp1 was not complexed with Beclin-1 under these experimental conditions.

3.6. The cytoprotective effects of miR-221 were blocked by over-expression of Ddit4 or Tp53inp1 but not p27 or Bak1

To validate whether Ddit4 and Tp53inp1 were authentically involved in the protection of miR-221 against H/R, we observed the effect by restoration of the expressions of Ddit4 and Tp53inp1 in miR-221 transfected cells. pcDNA3.1 vectors of Ddit4, Tp53inp1, p27 and Bak1 were constructed to directly validate gene effect in H/R (S. 5b). LDH release was reduced by miR-221 with or without additional empty pcDNA3.1 vector transfection (Fig. 4 c). Additional empty pcDNA3.1 vector transfection did not alter the protective effect of miR-221. pcDNA3.1 vectors of Ddit4, Tp53inp1, p27 and Bak1 were co-transfected with miR-221. Compared with pcDNA3.1 empty vector, the protective effect of miR-221 was blocked by over-expression of Ddit4 and Tp53inp1, but not p27 or Bak1 (Fig. 4 d).

4. Discussion

Our data indicated that the cytoprotective effects of miR-221in H/R injury was through attenuation of autophagy. MiR-221 directly inhibited autophagosome formation by targeting Ddit4 (thus disinhibiting the mTORC1/p-4EBP4 pathway) and autophagosome degradation by targeting Tp53inp1 (reducing p62 complex formation). Therefore, miR-221 is a potential therapeutic target in the amelioration of I/R injury.

Ddit4, also known as RTP801 and REDD1, was induced by either HIF-1 or DNA damage [21,22]. Ddit4 is rapidly up-regulated in response to multiple stresses and facilitates apoptosis and autophagy in tumor cells and in neurodegeneration [16,23,24]. Ddit4 was found to play a role in alcoholic skeletal myopathy and muscle atrophy due to mTORC1 regulated autophagy [25,26]. Little is known of its role in the heart. One study reported that Ddit4 was up-regulated in a cardiac I/R model [27], but no mechanism was demonstrated. Our previous study linked miR-221 with Ddit4 [16]. In this study, Luciferase reporter study confirmed Ddit4 as a target of miR-221. Since there are strong evidences indicate that Ddit4 inhibit mTORC1 in associated with cell death [7,24,28], we further examined the effect of miR-221 on mTORC1/4EBP1 and p70s6k signaling pathway. MiR-221 reversed inhibition of the mTORC1 signaling pathway caused by H/R and Rapamycin as indicated by increases of p-mTOR and p-4EBP1. MiR-221 reduced Rapamycin-induced cell injury as well. The converse decrease in p-mTOR and p-4EBP1 by miR-221-i corroborated these findings. The overall changes of p70s6k caused by miR-221 or miR-221-i were relatively weaker compared to p-4EBP1. These results are consistent with a report that mTORC1 through 4EBP1 regulates cardiac function and myocardium survival in response to stress [29]. All together, our data demonstrate that targeting of Ddit4 is one mechanism underpinning the protective effect of miR-221 in H/R injury. A similar mechanism has been reported in liver tumorigenesis [30].

Tp53inp1 is a tumor suppressor but it is also expressed in many tissues and induced by various stresses [31]. Previous work has demonstrated that stress-induced up-regulation of Tp53inp1 is dependent upon p53 activation [32,33]. Our data suggested miR-221 may directly target Tp53inp1. P53 was not the upstream of Tp53inp1 but could be downstream in this setting (data not shown). Due to limitations imposed by the incomplete rat Tp53inp1 3′-UTR sequence available, we could not construct a plasmid to perform Luciferase reporter assay to fully validate Tp53inp1 as a target of miR-221. However, based on reciprocal

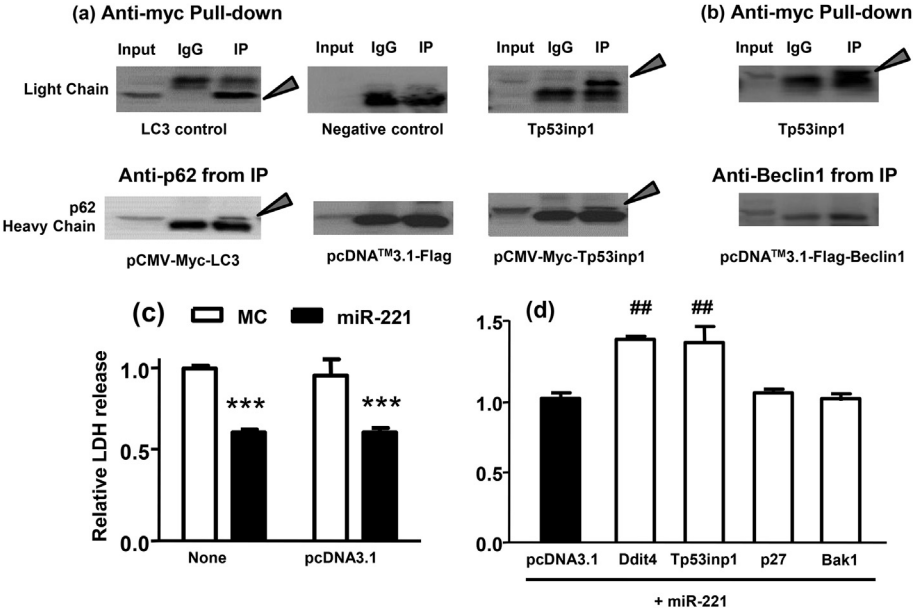

Fig. 4. Co-IP to detect complexes of LC3/p62, Tp53inp1/p62 formation and target gene recovery study. (a) Upper: anti-Myc pull down; lower: anti-p62 indicating p62 was detected from anti-Myc pull-down. (b) Upper: anti-Myc pull down; lower: anti-Flag to detect Beclin1. (c) miR-221 mimic inhibited H/R-induced LDH release without or with additional pcDNA 3.1 vector transfection. (d) Decrease of LDH release caused by miR-221 was diminished by over-expressions of Ddit4 or Tp53inp1. **p < 0.01 vs. MC; ##p < 0.01 vs. Cont by two-tail unpaired t-test. N = 3 in triplicate.

down- and up-regulation of Tp53inp1 by miR-221 mimic and inhibitor respectively, we suspect Tp53inp1 is a target of miR-221. Since Tp53inp2 shares 45% similarity with Tp53inp1 [34], it was tested as well in this study. Luciferase reporter assays did not confirm Tp53inp2 being a target of miR-221. Therefore, we focused on Tp53inp1 to demonstrate its role in H/R, which to our knowledge, has not been previously reported.

P62 is an important adapter modulating degradation of ubiquitinated proteins during autophagy [10]. The level of p62 usually correlates inversely with protein degradation in autophagy. MiR-221 increased p62 levels consistent with inhibition of autophagy. MiR-221-i evoked the opposite effect. The unique structure of p62 which contains six main domains, makes it an inter-player between autophagy and multiple signaling pathways [10]. The p62 C-terminal domain links to ubiquitinated protein to promote their selective degradation of specific proteins. The LC3-interacting domain links to LC3 to form an autophagosome-lysosome. The formation of a complex of p62/LC3 was previously reported in tumorigenesis [35]. It was re-confirmed in our study. Our identification of Tp53inp1/p62 complex formation is novel. A similar approach was taken to demonstrate the interaction between Tp53inp1 and Beclin-1. With Myc-Tp53inp1 pull-down, Beclin-1 could not be detected. In summary, by targeting Tp53inp1, miR-221 reduced Tp53inp1/p62 complex formation resulting in inhibition of H/R-induced autophagy.

The effects of miR-221 targeting on Ddit4 and Tp53inp1 were well supported by our results of GFP-LC3 autophagosome detection, LC3-I, -II and ratio, and p62. LC3-I is localized in the cytoplasm and LC3-II is associated with outer and inner membranes of autophagosome [19]. They were visualized as a diffused fluorescence (Fig. 2 a Nor) and as punctuate structures (Fig. 2 a I/R, Rap and MC) under microscopy. I/R-induced autophagosome formation was significantly reduced by miR-221 as shown the reduction of puncta-positive cells from 50% to 20% (I/R or MC vs. miR-221). In addition, the LC3-II/I ratio was reduced. The levels of LC3-I and II are a result of the conjugation of LC3 occurring during autophagosome formation and the degradation of conjugated LC3 after the fusion of autophagosome and lysosome, termed as autophagy flux [36]. The measurement of LC3-I and −II is not sufficient to determine the extent of functional autophagy. Bafilomycin A (Baf), an inhibitor of lysosome degradation, was applied to measure autophagy flux. Significant increases in LC3 II and II/I ratios indicated the inhibition of autophagosome degradation. Greatly elevated LC3-I levels with or without Baf further supported miR-221 inhibition of autophagosome formation. Notably miR-221 increased LC3-II level with or without Baf which was an evidence of inhibition of autophagosome degradation. It was further supported by the increases of p62 [11,19]. Therefore miR-221 has dual effects inhibiting both autophagosome formation and degradation through targeting on Ddit4 and Tp53inp1 respectively.

5. Conclusions

Through modulation of autophagy, miR-221 is a promising therapeutic target in H/R. These cardioprotective actions may occur through direct targeting on Ddit4 and Tp53inp1 which disinhibits the mTORC1/p-4EBP1 pathway and reduces Tp53inp1/p62 complex formation. These results require corroboration in experimental in vivo cardiac I/R.

Acknowledgments

Plasmid pEGFP-LC3 was a kind gift from Dr. Jianghua Li (Key Laboratory of Medical Molecular Virology, Shanghai Medical College, Fudan University, Shanghai, China). miRVec vector were kindly provided by Dr. Leah Vardy (Institute of Medical Biology, A*STAR, Singapore). This work was supported by grants awarded to Professor A Mark Richards including the Cardiovascular Research Institute Start-up Fund (National University of Singapore, Singapore) and a National Medical Research Council Center Grant (Ministry of Health -Singapore).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2016.04.090.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2016.04.090.

References

[1] R. Scherz-Shouval, E. Shvets, E. Fass, H. Shorer, L. Gil, Z. Elazar, Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4, EMBO J. 26 (2007) 1749–1760.
[2] P. Bernardi, A. Rasola, Calcium and cell death: the mitochondrial connection, Subcell. Biochem. 45 (2007) 481–506.
[3] Y. Matsui, H. Takagi, X. Qu, M. Abdellatif, H. Sakoda, T. Asano, B. Levine, J. Sadoshima, Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy, Circ. Res. 100 (2007) 914–922.
[4] H. Zhang, M. Bosch-Marce, L.A. Shimoda, Y.S. Tan, J.H. Baek, J.B. Wesley, F.J. Gonzalez, G.L. Semenza, Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia, J. Biol. Chem. 283 (2008) 10892–10903.
[5] X. Ma, H. Liu, S.R. Foyil, R.J. Godar, C.J. Weinheimer, J.A. Hill, A. Diwan, Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury, Circulation 125 (2012) 3170–3181.
[6] T. Hara, A. Takamura, C. Kishi, S. Iemura, T. Natsume, J.L. Guan, N. Mizushima, FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells, J. Cell Biol. 181 (2008) 497–510.
[7] M.D. Dennis, N.K. McGhee, L.S. Jefferson, S.R. Kimball, Regulated in DNA damage and development 1 (REDD1) promotes cell survival during serum deprivation by sustaining repression of signaling through the mechanistic target of rapamycin in complex 1 (mTORC1), Cell Signal 25 (2013) 2709–2716.
[8] S. Mukhopadhyay, P.K. Panda, N. Sinha, D.N. Das, S.K. Bhutia, Autophagy and apoptosis: where do they meet? Apoptosis 19 (2014) 555–566.
[9] Y. Kabeya, N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, T. Yoshimori, LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing, EMBO J. 19 (2000) 5720–5728.
[10] M. Lippai, P. Low, The role of the selective adaptor p62 and ubiquitin-like proteins in autophagy, Biomed. Res. Int. 2014 (2014) 832704.
[11] M. Komatsu, S. Waguri, M. Koike, Y.S. Sou, T. Ueno, T. Hara, N. Mizushima, J. Iwata, J. Ezaki, S. Murata, J. Hamazaki, Y. Nishito, S. Iemura, T. Natsume, T. Yanagawa, J. Uwayama, E. Warabi, H. Yoshida, T. Ishii, A. Kobayashi, M. Yamamoto, Z. Yue, Y. Uchiyama, E. Kominami, K. Tanaka, Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice, Cell 131 (2007) 1149–1163.
[12] E. van Rooij, E.N. Olson, MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets, J. Clin. Invest. 117 (2007) 2369–2376.
[13] R.S. Pillai, S.N. Bhattacharyya, C.G. Artus, T. Zoller, N. Cougot, E. Basyuk, E. Bertrand, W. Filipowicz, Inhibition of translational initiation by Let-7 MicroRNA in human cells, Science 309 (2005) 1573–1576.
[14] M.P. Perron, P. Provost, Protein interactions and complexes in human microRNA biogenesis and function, Front. Biosci. 13 (2008) 2537–2547.
[15] V. Sala, S. Bergerone, S. Gatti, S. Gallo, A. Ponzetto, C. Ponzetto, T. Crepaldi, MicroRNAs in myocardial ischemia: identifying new targets and tools for treating heart disease. New frontiers for miR-medicine, Cell Mol. Life Sci. 71 (2014) 1439–1452.
[16] Y. Zhou, Q. Chen, K.S. Lew, A.M. Richards, P. Wang, Discovery of potential therapeutic miRNA targets in Cardiac Ischemia-reperfusion injury, J. Cardiovasc Pharmacol. Ther. 21 (3) (2015) 296–309.
[17] M. Su, J. Wang, C. Wang, X. Wang, W. Dong, W. Qiu, Y. Wang, X. Zhao, Y. Zou, L. Song, L. Zhang, R. Hui, MicroRNA-221 inhibits autophagy and promotes heart failure by modulating the p27/CDK2/mTOR axis, Cell Death Differ. 22 (6) (2014) 986–999.
[18] J. Li, Y. Liu, Z. Wang, K. Liu, Y. Wang, J. Liu, H. Ding, Z. Yuan, Subversion of cellular autophagy machinery by hepatitis B virus for viral envelopment, J. Virol. 85 (2011) 6319–6333.
[19] N. Mizushima, T. Yoshimori, B. Levine, Methods in mammalian autophagy research, Cell 140 (2010) 313–326.

[20] S.Y. Park, J.H. Lee, M. Ha, J.W. Nam, V.N. Kim, miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42, Nat. Struct. Mol. Biol. 16 (2009) 23–29.
[21] T. Shoshani, A. Faerman, I. Mett, E. Zelin, T. Tenne, S. Gorodin, Y. Moshel, S. Elbaz, A. Budanov, A. Chajut, H. Kalinski, I. Kamer, A. Rozen, O. Mor, E. Keshet, D. Leshkowitz, P. Einat, R. Skaliter, E. Feinstein, Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis, Mol. Cell Biol. 22 (2002) 2283–2293.
[22] L.W. Ellisen, K.D. Ramsayer, C.M. Johannessen, A. Yang, H. Beppu, K. Minda, J.D. Oliner, F. McKeon, D.A. Haber, REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species, Mol. Cell 10 (2002) 995–1005.
[23] L.M. Knowles, C. Yang, A. Osterman, J.W. Smith, Inhibition of fatty-acid synthase induces caspase-8-mediated tumor cell apoptosis by up-regulating DDIT4, J. Biol. Chem. 283 (2008) 31378–31384.
[24] M. Canal, J. Romani-Aumedes, N. Martin-Flores, V. Perez-Fernandez, C. Malagelada, RTP801/REDD1: a stress coping regulator that turns into a troublemaker in neurodegenerative disorders, Front. Cell Neurosci. 8 (2014) 313.
[25] F.A. Britto, G. Begue, B. Rossano, A. Docquier, B. Vernus, C. Sar, A. Ferry, A. Bonnieu, V. Ollendorff, F.B. Favier, REDD1 deletion prevents dexamethasone-induced skeletal muscle atrophy, Am. J. Physiol. Endocrinol. Metab. 307 (2014) E983–E993.
[26] C.H. Lang, R.A. Frost, T.C. Vary, Acute alcohol intoxication increases REDD1 in skeletal muscle, Alcohol Clin. Exp. Res. 32 (2008) 796–805.
[27] M.Y. Kim, E.J. Seo, D.H. Lee, E.J. Kim, H.S. Kim, H.Y. Cho, E.Y. Chung, S.H. Lee, E.J. Baik, C.H. Moon, Y.S. Jung, Gadd45beta is a novel mediator of cardiomyocyte apoptosis induced by ischaemia/hypoxia, Cardiovasc Res. 87 (2010) 119–126.
[28] Y. Zhao, X. Xiong, L. Jia, Y. Sun, Targeting Cullin-RING ligases by MLN4924 induces autophagy via modulating the HIF1-REDD1-TSC1-mTORC1-DEPTOR axis, Cell Death Dis. 3 (2012) e386.
[29] D. Zhang, R. Contu, M.V. Latronico, J. Zhang, R. Rizzi, D. Catalucci, S. Miyamoto, K. Huang, M. Ceci, Y. Gu, N.D. Dalton, K.L. Peterson, K.L. Guan, J.H. Brown, J. Chen, N. Sonenberg, G. Condorelli, MTORC1 regulates cardiac function and myocyte survival through 4E-BP1 inhibition in mice, J. Clin. Invest. 120 (2010) 2805–2816.
[30] P. Pineau, S. Volinia, K. McJunkin, A. Marchio, C. Battiston, B. Terris, V. Mazzaferro, S.W. Lowe, C.M. Croce, A. Dejean, miR-221 overexpression contributes to liver tumorigenesis, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 264–269.
[31] J. Shahbazi, R. Lock, T. Liu, Tumor protein 53-induced nuclear protein 1 enhances p53 function and represses tumorigenesis, Front. Genet. 4 (2013) 80.
[32] R. Tomasini, A.A. Samir, M.J. Pebusque, E.L. Calvo, S. Totaro, J.C. Dagorn, N.J. Dusetti, J.L. Iovanna, P53-dependent expression of the stress-induced protein (SIP), Eur. J. Cell Biol. 81 (2002) 294–301.
[33] S. Okamura, H. Arakawa, T. Tanaka, H. Nakanishi, C.C. Ng, Y. Taya, M. Monden, Y. Nakamura, p53DINP1, a p53-inducible gene, regulates p53-dependent apoptosis, Mol. Cell 8 (2001) 85–94.
[34] A. Sancho, J. Duran, A. Garcia-Espana, C. Mauvezin, E.A. Alemu, T. Lamark, M.J. Macias, R. DeSalle, M. Royo, D. Sala, J.U. Chicote, M. Palacin, T. Johansen, A. Zorzano, DOR/Tp53inp2 and Tp53inp1 constitute a metazoan gene family encoding dual regulators of autophagy and transcription, PLoS One 7 (2012) e34034.
[35] R. Mathew, C.M. Karp, B. Beaudoin, N. Vuong, G. Chen, H.Y. Chen, K. Bray, A. Reddy, G. Bhanot, C. Gelinas, R.S. Dipaola, V. Karantza-Wadsworth, E. White, Autophagy suppresses tumorigenesis through elimination of p62, Cell 137 (2009) 1062–1075.
[36] S.T. Shibutani, T. Yoshimori, A current perspective of autophagosome biogenesis, Cell Res. 24 (2014) 58–68.