PTEN-mediated mitophagy and APE1 overexpression protects against cardiac hypoxia/reoxygenation injury

Wenshuai Tang[1] · Deqing Lin[1] · Mingxiang Chen[1] · Zhiping Li[1] · Weimin Zhang[1] · Wenping Hu[1] · Fuping Li[1]

Abstract

Autophagy plays a critical role in cardiac hypoxia/reoxygenation (H/R). Studies indicated that the phosphatase and tensin homolog (PTEN) influences level of autophagy. This study aims to explore the role of PTEN mediating a specific autophagy, mitophagy, in cardiac H/R injury. H9c2 cells were cultured and suffered hypoxia and reoxygenation treatment. To inhibit function of PTEN protein, bpv (phen) was added into medium throughout the process of H/R injury. In addition, we overexpressed the apurinic/apyrimidinic endonuclease 1 (APE1) in H/R-injured H9c2 cells. Then the cell viability, apoptosis, and release of Cytochrome C were determined through CCK-8 assay, flow cytometry, and western blotting, respectively. The results indicated that H/R significantly induced autophagy, as identified by an increased level of microtubule-associated protein 1 light chain 3 beta (LC3B) and a decreased level of sequestosome 1 (P62). After stimulation of bpv (phen), PTEN-induced putative kinase protein 1 (PINK1)/Parkin-mediated mitophagy was inhibited, while apoptosis and releases of Cytochrome C were both significantly increased, indicating an exacerbated H/R injury. Furthermore, the overexpression of APE1 attenuated the apoptosis and releases of Cytochrome C induced by H/R injury, and promoted PINK1/Parkin-mediated mitophagy. Our findings provide an insight into the PTEN and APE1 overexpression protects against cardiac hypoxia/reoxygenation injury, which may be through inducing the PINK1/Parkin-mediated mitophagy.

Keywords Mitophagy · Hypoxia/reoxygenation · Cardiomyocyte apoptosis · PTEN

Introduction

Myocardial infarction (MI) is a cardiovascular disease which is mainly characterized by myocardial necrosis caused by acute or persistent ischemia and anoxia. Although survival following a first myocardial infarct approaches 90% today, re-infarction occurs commonly and carries a high mortality (Dutta e*t al.* 2012). In a population study, 1-yr risk of re-infarction reached 17.4% (Christos e*t al.* 2010). Timely reperfusion after the onset of ischemia is the current standard therapy for myocardial ischemia (Gerczuk and Kloner 2012; Members e*t al.* 2013). However, reperfusion strategies may result in another unpleasant injury, ischemia/reperfusion (I/R) (Yellon and Hausenloy 2007; Hausenloy and Yellon 2015). Reperfusion injury is mainly caused by some cellular alterations such as oxidative stress and inflammation (Gong e*t al.* 2013; Braunwald 2015). Thus, theoretical research on preventing myocyte death following I/R is necessary to develop the novel strategies on treating patients with MI.

Oxidative stress induced by I/R always related to autophagy in cardiomyocyte (Hariharan e*t al.* 2011). Recent studies have shown that levels of autophagy were enhanced in the I/R-injured cardiomyocyte (Jian e*t al.* 2011; Przyklenk e*t al.* 2012). However, a study has reported that autophagy has a dual, opposite role in the heart, depending on the stimulus (Gustafsson and Gottlieb 2009). There is general agreement that upregulation of autophagy is cardioprotective and profoundly attenuates myocardial ischemia/reperfusion injury (Kanamori e*t al.* 2011; Loos e*t al.* 2011; Cui e*t al.* 2014). But excessive autophagy also could induce apoptosis of cardiomyocyte (Huang e*t al.* 2015; Huang e*t al.* 2017).

Mitophagy, the specific autophagic elimination of mitochondria, is regulated in many metazoan cell types by *Parkin* and *PINK1* (Youle and Narendra 2011). Parkin protein deficiency could promote cardiac injury and reduce mice survival following myocardial infarction (Kubli e*t al.* 2013). *PINK1* played as a cardioprotective kinase (Siddall e*t al.* 2008), and a study has reported that *PINK1*-mediated

✉ Fuping Li
651023@hospital.cqmu.edu.cn

[1] Department of Heart and Vascular Surgery, Cardiovascular Disease Center, The Third Affiliated Hospital of Chongqing Medical University, No. 1, Shuang Hu Branch Road, Hui Xing street, Yubei District, Chongqing 401120, China

autophagy was regulated by *PTEN* (Roe e*t al.* 2015). Robust evidence also proved *PTEN* plays an important role in autophagy (Ueno e*t al.* 2008). PTEN nuclear translocation could promote autophagy in response to DNA damage (Chen et al. 2015a). However, little was known about *PTEN* regulating mitophagy in I/R-injured cardiomyocyte.

APE1, an oxidative repair protein, can transfer from nuclear to mitochondrion. Reactive oxygen species (ROS) could induce the activation of APE1 (Pines e*t al.* 2005). Its main function is repairing spontaneous DNA damage (Whitaker e*t al.* 2018), and this function also existed in mitochondrion (Li e*t al.* 2010). However, it remains unclear whether *APE1* influence mitochondrial function during I/R injuring in cardiomyocyte.

In this study, we cultured H9c2 cells and conducted hypoxia/ reoxygenation (H/R) treatment. We showed that inhibiting of *PTEN* function and *APE1* overexpression may play a regulatory role in mitophagy, which may be regulate number of mitochondrion and maintain its quality control. *PTEN*-mediated mitophagy may protect against cardiac H/R injury.

Materials and Methods

Cell culture H9c2 cells (rat cardiomyocytes) were purchased from American Type Culture Collection (ATCC, Manassas, VA) Cells resuscitated from liquid nitrogen refrigeration were cultured in high glucose-Dulbecco Minimum Essential Medium (H-DMEM) (Solarbio, Beiking, China) with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA) and 1% streptomycin/penicillin, at 37°C with 5% CO_2.

Hypoxia/reoxygenation Cells were cultured with H-DMEM/ serum-free starvation, 2 h before exposure to hypoxia. To conduct hypoxia, H9c2 cells were placed in a 37°C airtight chamber saturated with 95% N_2/5% CO_2 for 24 h. Reoxygenation was performed in a 37°C/5% CO_2 incubator under H-DMEM/10% FBS for 30 min (Webster e*t al.* 1999). To inhibit function of PTEN protein, 5 μM of bpv (phen) (Enzo Life Sciences, Farmingdale, NY) was added in the above process. After H/R treatment, cells were harvested and used in other assays.

Cell transfection To overexpress *APE1* in H9c2 cells, the coding sequence of *APE1* was cloned through primers (forward: 5′-GGG GTA CCA TGC CGA AGC GTG GGA AAA AG-3′; reverse: 5′-CCG CTC GAG TCA CAG TGC TAG GTA TAG GGT GAT AGG AC-3′) and inserted into the expression plasmid pcDNA3.0 (Invitrogen, Carlsbad, CA). Recombinant plasmid was transfected into H9c2 cells through Lipofectamine 2000 Reagent (Invitrogen). Cells transfected with the wild-type plasmid pcDNA3.0 were used as control.

At 24 h after transfection, cells were harvested and used to perform H/R treating.

Cell activity assay Equal numbers of H9c2 cells (1×10^4) suffered H/R treating in each group. Subsequently, cells were washed 3 times and resuspended by phosphate buffer saline. Then cell activities were detected through the Cell Counting Kit-8 (Dojindo, Japan). Briefly, 100 μl of CCK-8 solution was added into each well and then it was incubated for 4 h at 37°C with 5% CO_2. Finally, the optical density at 450 nm was recorded by a microplate reader (Thermo Scientific).

Flow cytometry The apoptosis induced by H/R injury was measured by flow cytometry assay using an Annexin V-FITC/PI Apoptosis Kit (MultiSciences, Hangzhou, China). The H/R-treated cells (5×10^5) were washed 3 times by phosphate buffer saline and moved to flow tubes after trypsin digestion. Then they were resuspended by 200 μl of Annexin V-FITC/PI solution and incubated for 15 min at dark. Finally, the fluorescence signal value was measured by a BD FACS Calibur™ flow cytometry (BD Biosciences, Franklin, NJ).

Hypoxia/reoxygenation Cells were cultured with H-DMEM/ serum-free starvation, 2 h before exposure to hypoxia. To conduct hypoxia, H9c2 cells were placed in a 37°C airtight chamber saturated with 95% N_2/5% CO_2 for 24 h. Reoxygenation was performed in a 37°C/5% CO_2 incubator under H-DMEM/10% FBS for 30 min (Webster e*t al.* 1999). To inhibit function of PTEN protein, 5 μM of bpv (phen) (Enzo Life Sciences, Farmingdale, NY) was added in the above process. After H/R treatment, cells were harvested and used in other assays.

Autophagy detection The level of autophagy in the H/R-treated H9c2 cells was detected by the Ad-GFP-LC3B, adenovirus expressing GFP-LC3B fusion protein (Beyotime, Shanghai, China). Briefly, cells (1×10^5) were transfected with Ad-GFP-LC3B (20 MOI) at 24 h before the H/R treating. After H/R treating, the green fluorescence signal was visualized by a Leica DMI6000 B Microscope (Leica, Germany). Furthermore, we used the mt-Keima assay (Bingol e*t al.* 2014) to observe the mitophagy in the H9c2 cells. Briefly, cells (1×10^5) were transfected with lentivirus-packaged mt-Keima-COX8 (50 MOI), which leads to overexpression of Keima protein in mitochondrion. As the Keima protein releases different fluorescence signals under acidic and neutral conditions, the mitophagy can be observed when the mitochondrial autophagy is fused with acid lysosome. Twenty-four hours after transfection, cells suffered H/R treating or other administration. Finally, the green fluorescence signal (458 nm) and red fluorescence signal (543 nm) were visualized by a confocal microscopy (TCS SP8 X, Leica, Germany).
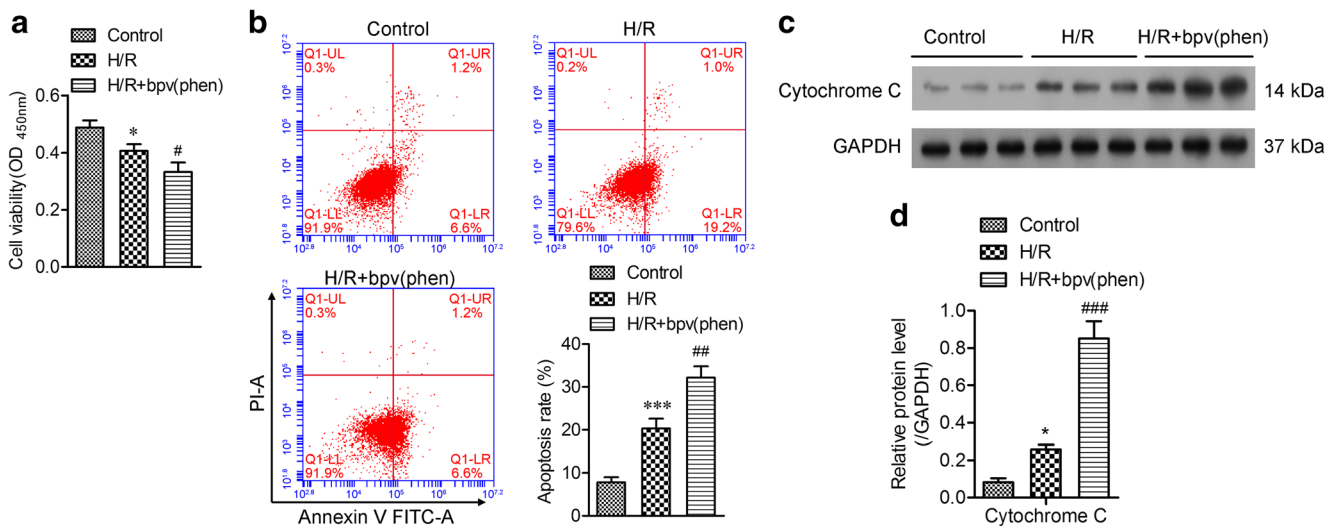
Figure 1. Bpv (phen) exacerbated cardiac H/R injury. H/R-injured H9c2 cells were simulated with 5 μM of bpv (phen). (*A*) Result of CCK-8 assay showed bpv (phen) caused a further decrease of cell viability. (*B*) Bpv (phen) increased the apoptosis of H/R-injured H9c2 cells. (*C, D*) Western blot analysis with cytoplasmic protein showed an increased release of Cytochrome C in H/R + bpv (phen) group. GAPDH was used as reference protein. $*P < 0.05$ and $***P < 0.001$ *vs.* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ *vs.* H/R.

Western blotting Cell total proteins were extracted with lysis buffer containing 50 mM Tris (pH 7.6), 150 mM NaCl, 1% TritonX-100, 1% deoxycholate, 0.1% SDS, 1 mM PMSF, and 0.2% aprotinin (Sigma-Aldrich, Saint Louis, MO). The cytoplasmic and mitochondrial protein was extracted through a Cytoplasmic and Mitochondrial Protein Extraction Kit (Sangon Biotech, Shanghai, China). The concentrations of extracted protein were determined with the BCA Protein Assay kit (Beyotime). Then 20-μg protein samples were separated by a SDS-PAGE electrophoresis and transferred to a polyvinylidene fluoride membrane. The membrane was blocked with 5% evaporated skimmed milk tris-buffered saline (TBS) at room temperature overnight; washed 3 times by TBS; and subsequently incubated with anti-Cytochrome C (Abcam, diluted 1:800), anti-APE1 (Abcam, diluted 1:2000), anti-PTEN (Abcam, diluted 1:400), anti-PINK1 (Abcam, diluted 1:500), anti-Parkin (Abcam, diluted 1:400), anti-LC3 (Abcam, diluted 1:1500), or anti-P62 (Abcam, diluted 1:1000) at 4°C overnight. GAPDH (Abcam, diluted 1:5000) and COX (Abcam, diluted 1:1000) were used as an internal control, washed 3 times again, and incubated with HRP Goat anti-Rabbit IgG (BOSTER, Wuhan, China) for 40 min. Finally, the protein bands were visualized with ECL-Plus reagent (Millipore, Billerica, MA) and scanned using a bio-imaging analyzer (Bio-Rad, Hercules, CA).

Statistical analysis All data were obtained from at least three independent experiments and represented as mean ± standard deviation. An ANOVA analysis with Tukey's multiple comparisons test was used to analyze differences between multigroups in GraphPad Prism 6 (GraphPad Software, San Diego, CA). *P* values < 0.05 were considered statistically significant.

Results

Loss of *PTEN* function promotes cardiac H/R injury As *PTEN* plays a promotion role in autophagy (Arico e*t al.* 2001; Ueno e*t al.* 2008), this study initially aimed to explore its role in the H/R-injured cardiomyocyte. To inhibit function of PTEN protein, 5 μM of bpv (phen) was added into medium throughout the process of H/R injury. CCK-8 detection found that the cell viability in the H/R group is significantly decreased compared with the control group, and in the H/R + bpv (phen) group, it is also significantly lower than that in the H/R group (Fig. 1*A*). In addition, results of flow cytometry assay showed that H/R treating significantly promotes apoptosis of H9c2 cells and this effect is significantly enhanced by stimulation of bpv (phen), especially 19.3% of early apoptosis in the H/R group increased to 31.9% in the H/R + bpv (phen) group (Fig. 1*B*). As release of Cytochrome C from mitochondria induced caspase activation and apoptosis (Jiang and Wang 2004), we measured protein level of Cytochrome C in H/R-treated H9c2 cells by western blotting. Result showed that protein level of cytoplasmic Cytochrome C in the H/R group is significantly higher than that in the control group, and the stimulation with bpv (phen) significantly promotes the accumulation of cytoplasmic Cytochrome C (Fig. 1*C*, *D*). These results suggested that loss of *PTEN* function promotes the apoptosis and release of Cytochrome C from mitochondria in H/R-injured H9c2 cells.

Stimulation with bpv (phen) inhibits mitophagy in H/R-injured H9c2 cells To illuminate whether bpv (phen)
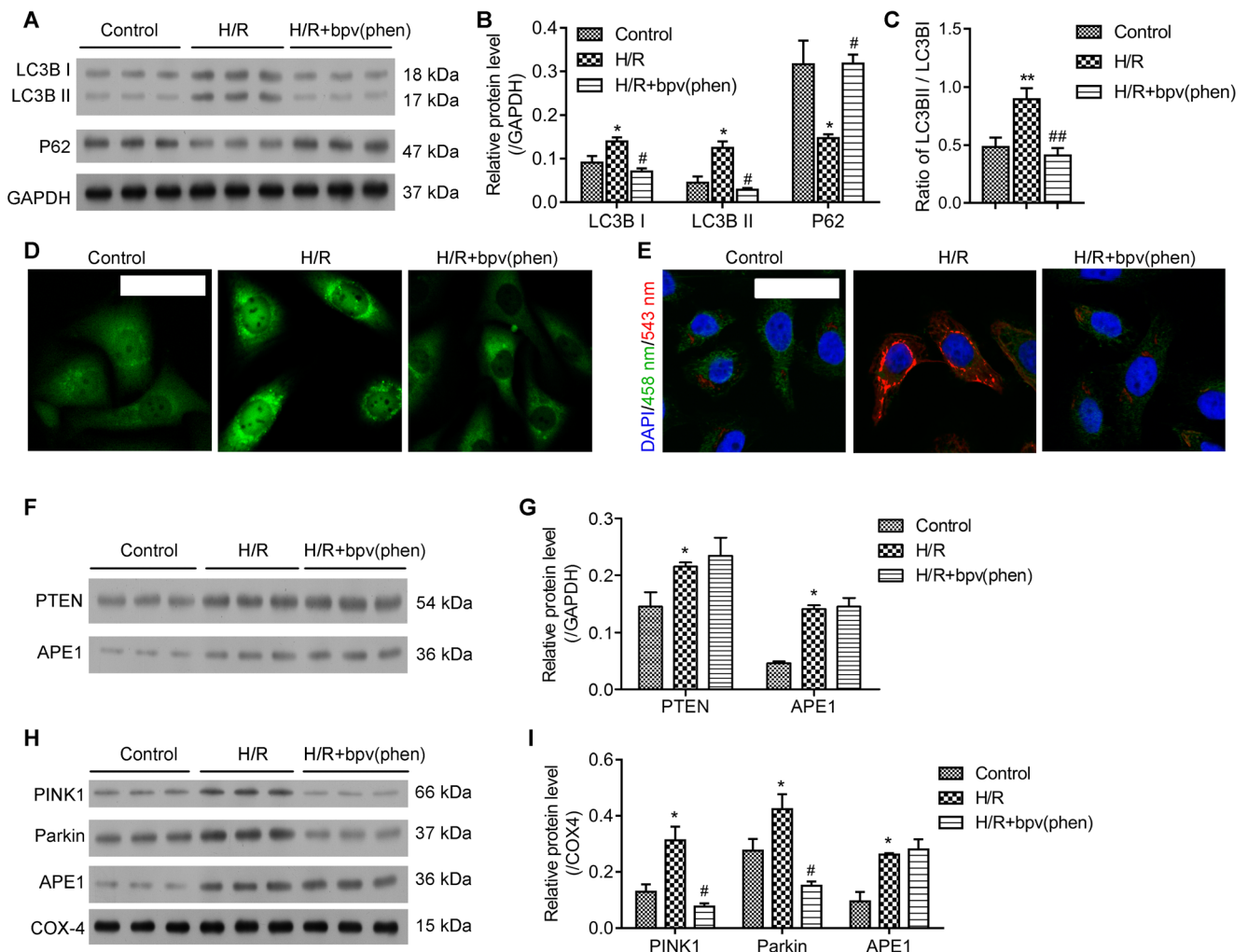
Figure 2. Bpv (phen) inhibited PINK1/Parkin-mediated mitophagy in H/R-injured H9c2 cells. H/R-injured H9c2 cells were simulated with 5 μM of bpv (phen). (A, B) Western blot analysis with cytoplasmic protein. Bpv (phen) significantly decreased the level of LC3B I and LC3B II, while increased level of P62. (C) The ratio of LC3B II/LC3B I. (D) The inhibited autophagy in bpv (phen)-treated group was determined with Ad-GFP-LC3B. *Scale bar* = 20 μm. (E) The mitophagy was shown by mt-Keima assay. *Scale bar* = 20 μm. (F, G) Western blot analysis with mitochondrial protein. The level change of total PTEN and APE1. (H, I) Western blot analysis showed the level change of mitochondrial PINK1, Parkin, and APE1. GAPDH and COX-4 were used as reference protein. *$P < 0.05$ *vs.* control; #$P < 0.05$ *vs.* H/R.

influences the release of Cytochrome C from mitochondria by regulating mitophagy, we detected protein level of LC3B, a key factor indicating autophagy. Results showed that H/R treating significantly induced expression of LC3B proteins, including LC3B I, LC3B II, and the ratio of LC3B II/LC3B I, while they were significantly suppressed by the stimulation of bpv (phen), and it appeared the opposite change for the levels of P62, a substrate of autophagy (Fig. 2A–C). Meanwhile, results showed by the Ad-GFP-LC3B also proved that H/R treating increased the autophagy level of H9c2 cells, while bpv (phen) decreased it (Fig. 2E). The mitophagy showed by mt-Keima assay has a same tendency (Fig. 2D). Western blotting revealed that total levels of PTEN and APE1 were both significantly increased in the H/R group (Fig. 2F, G).

Protein of mitochondrial PINK1 and Parkin was both significantly accumulated in the H/R group and significantly reduced by the stimulation of bpv (phen) (Fig. 2H, I). Of note, the level of mitochondrial APE1 was only increased in the H/R group. These results suggested that loss of *PTEN* function induces a level decline of autophagy in H/R-injured H9c2 cells, which may be a potential reason for increased release of Cytochrome C from mitochondria.

Overexpression of *APE1* attenuates cardiac H/R injury Above results showed level of mitochondrial APE1 was changed in H/R-injured H9c2 cells. To explore the role of *APE1* in cardiac H/R injury, we overexpressed *APE1* in H9c2 cells. Then it was found that overexpression of *APE1* significantly increased the cell viability of H/R-injured H9c2 cells when

compared with the H/R group (transfected with blank empty vector) (Fig. 3A). The percentage of early apoptosis in *APE1*-overexpressed H9c2 cells was 11.4%, which was significantly lower than 20.9% of the H/R group (Fig. 3B). Overexpression of *APE1* attenuated the apoptosis induced by H/R injury in H9c2 cells. In addition, the bands of western blotting showed that overexpression of *APE1* also significantly decreased the protein level of cytoplasmic Cytochrome C (Fig. 3C, D). These revealed that *APE1* plays a protective role in cardiac H/R injury.

APE1 promotes PINK1/Parkin-mediated mitophagy in H/R-injured H9c2 cells Studies reported that *APE1* mainly functions as a DNA repair enzyme (Tell et *al.* 2009; Whitaker et *al.* 2018). In this study, we aimed to discuss its effect on mitophagy. In *APE1*-overexpressed and H/R-injured H9c2 cells, we found the protein level of LC3B II and the ratio of LC3B II/LC3B I was significantly increased compared with that of only H/R-injured H9c2 cells, while it appeared the opposite change for the levels of P62 (Fig. 4A–C). The increased autophagy level in *APE1*-overexpressed and H/R-injured H9c2 cells was also proved by the more quantity of fluorescent spots showed with the Ad-GFP-LC3B (Fig. 4D). The mitophagy showed a same tendency (Fig. 4E). Overexpression of *APE1* was subsequently validated by western blotting, which showed both total cell APE1 and mitochondrial APE1 were significantly increased (Fig. 4F–I). Meanwhile, PINK1 and Parkin, two related proteins involved in mitophagy, were also accumulated in mitochondrion in *APE1*-overexpressed cells. These means overexpression of *APE1* promotes the process of *PINK1/Parkin*-mediated mitophagy.

Discussion

Mitochondrial dysfunction is one of the damage induced by I/R or H/R injury. Mitochondria are the primary sites for oxidative stress within cardiac cells. In this study, we performed H/R injury treatment in H9c2 cells. In H/R-injured cells, Cytochrome C was accumulated and the apoptosis rate was increased. Release of Cytochrome C induced by H/R could activate caspase-dependent apoptosis (Zou et *al.* 1997). Furthermore, level of autophagy and protein levels of mitochondrial PINK1 and Parkin were increased after the injury. H/R-induced autophagy has been reported in previous studies (Zhang et *al.* 2012; Liu et *al.* 2016). Activated PINK1/Parkin signal pathway in H9c2 cells indicated that the specific autophagy, mitophagy, was also induced by H/R injury (Sun et *al.* 2015). Considering that upregulation of autophagy is cardioprotective in cardiac H/R injury (Kanamori et *al.* 2011; Loos et *al.* 2011), it can be speculated that activated mitophagy may be a mechanism of self-repair of H9c2 cells, which could play an important role in the clearance of dysfunctional mitochondria and consequent decrease of Cytochrome C release.

To inhibit the function of PTEN, we stimulated H9c2 cells with the bpv (phen). Subsequently, we found that bpv (phen) exacerbated the cardiac H/R injury. Apoptosis rate was significantly increased under the stimulation of bpv (phen) plus H/R injury. A study has reported that bpv (phen) induced apoptosis through influencing autophagy (Chen et al. 2015b). However, our results were inconsistent with the study of Tian et *al.* (Tian et al. 2012), and we speculated that it may be mainly because of the different H/R-processing time or different concentration of bpv (phen). In our study, results showed that bpv (phen) decreased the protein levels of mitochondrial PINK1 and Parkin. PINK1/
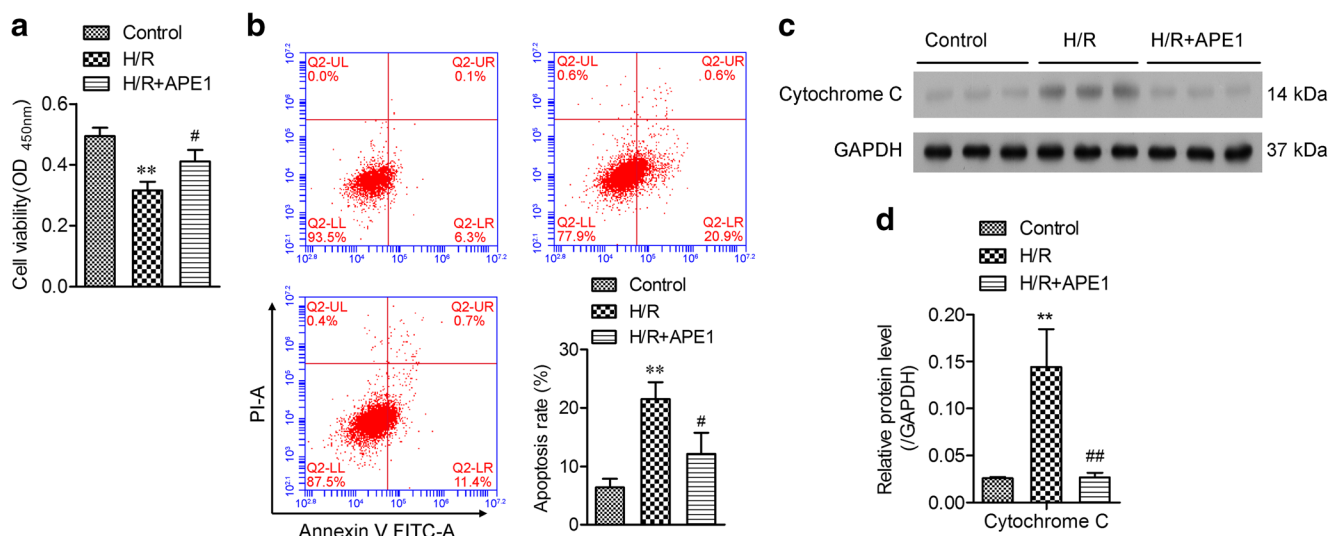

Figure 3. Overexpression of APE1 protected against cardiac H/R injury. (A) APE1-overexpressed H9c2 cells showed an increased cell viability. (B) Overexpression of APE1 inhibited apoptosis in H/R-injured H9c2 cells. (C, D) Western blot analysis with cytoplasmic protein showed an inhibited release of Cytochrome C in H/R + APE1 group. GAPDH was used as reference protein. **$P < 0.01$ vs. control; #$P < 0.05$ and ##$P < 0.01$ vs. H/R.
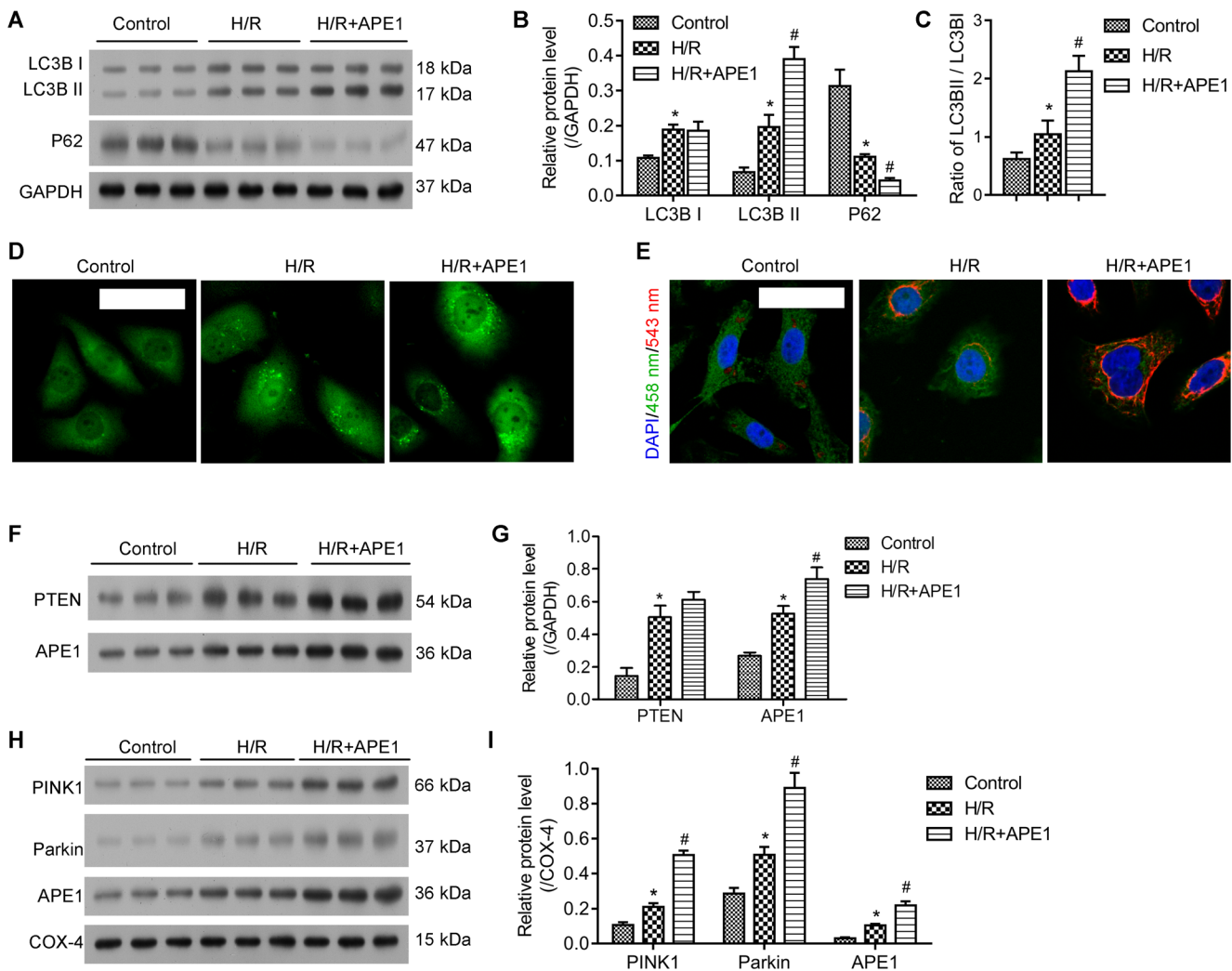
Figure 4. Overexpression of APE1 promoted PINK1/Parkin-mediated mitophagy in H/R-injured H9c2 cells. (A, B) Western blot analysis with cytoplasmic protein. In H/R-injured H9c2 cells, overexpression of APE1 significantly increased the level of LC3B I and LC3B II, while decreased the level of P62. (C) The ratio of LC3B II/LC3B I. (D) The enhanced autophagy in APE1 overexpression group was determined with Ad-GFP- LC3B. *Scale bar* = 20 μm. (E) The mitophagy was shown by mt-Keima assay. *Scale bar* = 20 μm. (F, G) Western blot analysis with mitochondrial protein. It showed the level change of total PTEN and APE1. (H, I) Western blot analysis showed the level change of mitochondrial PINK1, Parkin, and APE1. GAPDH and COX-4 were used as reference protein. *$P < 0.05$ *vs.* control; #$P < 0.05$ *vs.* H/R.

Parkin-mediated mitophagy was also inhibited by bpv (phen), which may subsequently induce the augment of dysfunctional mitochondria. The study of Yin *et al.* also proved that PTENα-deficiency impaired mitophagy-dependent mitochondrial clearance and exacerbated the cardiac H/R injury (Yin *et al.* 2018).

In the study, we found another mitochondrial protein, APE1, was also accumulated in H/R-injured H9c2 cells. APE1 overexpression partially decreased apoptosis rate and level of Cytochrome C. The author reported that APE1 can hinder the apoptosis of cardiac progenitor cells (Aonuma *et al.* 2016). Meanwhile, APE1 also overcome the multifunctional oxidative damage in nerve cells (Kaur *et al.* 2015). Of note, our results showed that APE1 overexpression also influenced the level of autophagy, which may be caused by the increase of mitochondrial PINK1 and Parkin. Parkin, a tumor

suppressor and Parkinson's disease (PD)–associated gene, has been found to play a role in maintaining the quality of mitochondrial APE1 (Scott *et al.* 2016). We suspected that the accumulation of multifunctional APE1 promoted the increase of mitochondrial Parkin, which was also shown by results of western blotting, and it subsequently induced high level of mitophagy. However, the mechanism of APE1 overexpression influencing autophagy still needs further experiments to explore.

Conclusion

In summary, our results found inhibition of PTEN function induced by bpv (phen)-suppressed PINK1/Parkin-mediated

mitophagy, which resulted in an increased apoptosis and release of mitochondrial Cytochrome C in H/R-injured H9c2 cells. Meanwhile, overexpression of APE1 also influenced the level of mitophagy and protected against cardiac hypoxia/reoxygenation injury. This study may provide a little theoretical basis for developing the novel strategies on treating patients with myocardial ischemia disease.

References

Aonuma T, Takehara N, Maruyama K, Kabara M, Matsuki M, Yamauchi A, Kawabe JI, Hasebe N (2016) Apoptosis-resistant cardiac progenitor cells modified with apurinic/apyrimidinic endonuclease/redox factor 1 gene overexpression regulate cardiac repair after myocardial infarction. Stem Cells Transl Med 5:1067–1078

Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, Ogierdenis E (2001) The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem 276:35243

Bingol B, Tea J, Phu L, Reichelt M, Bakalarski C, Song Q, Foreman O, Kirkpatrick D, Sheng M (2014) The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. Nature 510:370–375

Braunwald E (2015) The war against heart failure: the Lancet lecture. Lancet 385:812–824

Chen JH, Zhang P, Chen WD, Li DD, Wu XQ, Deng R, Jiao L, Li X, Ji J, Feng GK (2015b) ATM-mediated PTEN phosphorylation promotes PTEN nuclear translocation and autophagy in response to DNA-damaging agents in cancer cells. Autophagy 11:239–252

Chen Q, Yue F, Li W, Zou J, Xu T, Huang C, Zhang Y, Song K, Huang G, Xu G (2015a) Potassium bisperoxo(1,10-phenanthroline) oxovanadate (bpV(phen)) induces apoptosis and pyroptosis and disrupts the P62-HDAC6 protein interaction to suppress the acetylated microtubule-dependent degradation of autophagosomes. J Biol Chem 290:26051–26058

Christos M, Tomas J, Johan LC, Stefan A, Lars W, Ulf S (2010) Effect of angiotensin-converting enzyme inhibition on one-year mortality and frequency of repeat acute myocardial infarction in patients with acute myocardial infarction. Am J Cardiol 105:1229

Cui H, Li X, Li N, Qi K, Li Q, Jin C, Zhang Q, Jiang L, Yang Y (2014) Induction of autophagy by Tongxinluo through the MEK/ERK pathway protects human cardiac microvascular endothelial cells from hypoxia/reoxygenation injury. J Cardiovasc Pharmacol 64:180–190

Dutta P, Courties G, Wei Y, Leuschner F, Gorbatov R, Robbins C, Iwamoto Y, Thompson B, Carlson AL, Heidt T (2012) Myocardial infarction accelerates atherosclerosis. Nature 487:325–329

Gerczuk PZ, Kloner RA (2012) An update on cardioprotection : a review of the latest adjunctive therapies to limit myocardial infarction size in clinical trials. J Am Coll Cardiol 59:969–978

Gong G, Karamanlidis G, Chi FL, Tian R, Wang W (2013) Mitochondrial complex I deficiency promotes oxidative stress during ischemia reperfusion of cardiac myocytes. Circ Res A224

Gustafsson ÅB, Gottlieb RA (2009) Autophagy in ischemic heart disease. Circ Res 104:150–158

Hariharan N, Zhai P, Sadoshima J (2011) Oxidative stress stimulates autophagic flux during ischemia/reperfusion. Antioxid Redox Signal 14:2179–2190

Hausenloy DJ, Yellon DM (2015) Targeting myocardial reperfusion injury–the search continues. N Engl J Med 373:1073–1075

Huang Z, Han Z, Ye B, Dai Z, Shan P, Lu Z, Dai K, Wang C, Huang W (2015) Berberine alleviates cardiac ischemia/reperfusion injury by inhibiting excessive autophagy in cardiomyocytes. Eur J Pharmacol 762:1–10

Huang Z, Wu S, Kong F, Cai X, Ye B, Shan P, Huang W (2017) MicroRNA-21 protects against cardiac hypoxia/reoxygenation injury by inhibiting excessive autophagy in H9c2 cells via the Akt/mTOR pathway. J Cell Mol Med 21:467–474

Jian X, Xiao-Yan Z, Bin H, Yu-Feng Z, Bo K, Zhi-Nong W, Xin N (2011) MiR-204 regulate cardiomyocyte autophagy induced by hypoxia-reoxygenation through LC3-II. Int J Cardiol 148:110–112

Jiang X, Wang X (2004) Cytochrome C-mediated apoptosis. Annu Rev Biochem 73:87–106

Kanamori H, Takemura G, Goto K, Maruyama R, Ono K, Nagao K, Tsujimoto A, Ogino A, Takeyama T, Kawaguchi T (2011) Autophagy limits acute myocardial infarction induced by permanent coronary artery occlusion. Am J Physiol Heart Circ Physiol 300: 2261–2271

Kaur N, Dhiman M, Perez-Polo JR, Mantha AK (2015) Ginkgolide B revamps neuroprotective role of apurinic/apyrimidinic endonuclease 1 and mitochondrial oxidative phosphorylation against Aβ25-35-induced neurotoxicity in human neuroblastoma cells. J Neurosci Res 93:938–947

Kubli DA, Zhang X, Lee Y, Hanna RA, Quinsay MN, Nguyen CK, Jimenez R, Petrosyan S, Murphy AN, Gustafsson AB (2013) Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. J Biol Chem 288:915–926

Li M, Zhong Z, Zhu J, Xiang D, Dai N, Cao X, Qing Y, Yang Z, Xie J, Li Z (2010) Identification and characterization of mitochondrial targeting sequence of human apurinic/apyrimidinic endonuclease 1. J Biol Chem 285:14871–14881

Liu L, Wu Y, Huang X (2016) Orientin protects myocardial cells against hypoxia-reoxygenation injury through induction of autophagy. Eur J Pharmacol 776:90–98

Loos B, Genade S, Ellis B, Lochner A, Engelbrecht AM (2011) At the core of survival: autophagy delays the onset of both apoptotic and necrotic cell death in a model of ischemic cell injury. Exp Cell Res 317:1437–1453

Members WC, O'Gara PT, Kushner FG, Ascheim DD, Casey DE, Chung MK, Lemos JAD, Ettinger SM, Fang JC, Fesmire FM (2013) 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. J Am Coll Cardiol 61:e78–e140

Pines A, Perrone L, Bivi N, Romanello M, Damante G, Gulisano M, Kelley MR, Quadrifoglio F, Tell G (2005) Activation of APE1/Ref-1 is dependent on reactive oxygen species generated after purinergic receptor stimulation by ATP. Nucleic Acids Res 33: 4379–4394

Przyklenk K, Dong Y, Undyala VV, Whittaker P (2012) Autophagy as a therapeutic target for ischaemia/reperfusion injury? Concepts, controversies, and challenges. Cardiovasc Res 94:197

Roe ND, Xu X, Kandadi MR, Hu N, Pang J, Weiser-Evans MC, Ren J (2015) Targeted deletion of PTEN in cardiomyocytes renders cardiac contractile dysfunction through interruption of Pink1-AMPK signaling and autophagy. Biochim Biophys Acta 1852:290–298

Scott TL, Wicker CA, Suganya R, Dhar B, Pittman T, Horbinski C, Izumi T (2016) Polyubiquitination of apurinic/apyrimidinic endonuclease 1 by Parkin. Mol Carcinog 56:325

Siddall HK, Warrell CE, Davidson SM, Mocanu MM, Yellon DM (2008) Mitochondrial PINK1-a novel cardioprotective kinase? Cardiovasc Drugs Ther 22:507–508

Sun L, Zhao M, Yang Y, Xue RQ, Yu XJ, Liu JK, Zang WJ (2015) Acetylcholine attenuates hypoxia/reoxygenation injury by inducing mitophagy through PINK1/Parkin signal pathway in H9c2 cells. J Cell Physiol 231:1171–1181

Tell G, Quadrifoglio F, Tiribelli C, Kelley MR (2009) The many functions of APE1/Ref-1: not only a DNA repair enzyme. Antioxid Redox Signal 11:601–619

Tian Y, Daoud A, Shang J (2012) Effects of bpV(pic) and bpV(phen) on H9c2 cardiomyoblasts during both hypoxia/reoxygenation and H2O2-induced injuries. Mol Med Rep 5:852–858

Ueno T, Sato W, Horie Y, Komatsu M, Tanida I, Yoshida M, Ohshima S, Mak TW, Watanabe S, Kominami E (2008) Loss of Pten, a tumor suppressor, causes the strong inhibition of autophagy without affecting LC3 lipidation. Autophagy 4:692–700

Webster KA, Discher DJ, Kaiser S, Hernandez O, Sato B, Bishopric NH (1999) Hypoxia-activated apoptosis of cardiac myocytes requires reoxygenation or a pH shift and is independent of p53. J Clin Invest 104:239–252

Whitaker AM, Flynn TS, Freudenthal BD (2018) Molecular snapshots of APE1 proofreading mismatches and removing DNA damage. Nat Commun 9:1–11

Yellon DM, Hausenloy DJ (2007) Myocardial reperfusion injury. N Engl J Med 357:1121–1135

Yin Y, Li G, Yang J, Yang C, Zhu M, Jin Y, Mcnutt MA (2018) PTENα regulates mitophagy and maintains mitochondrial quality control. Autophagy 14:1742–1760

Youle RJ, Narendra DP (2011) Mechanisms of mitophagy. Nat Rev Mol Cell Biol 12:9–14

Zhang ZL, Fan Y, Liu ML (2012) Ginsenoside Rg1 inhibits autophagy in H9c2 cardiomyocytes exposed to hypoxia/reoxygenation. Mol Cell Biochem 365:243–250

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X (1997) Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c–dependent activation of caspase-3. Cell 90:405–413