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Autophagy activation promotes removal of damaged mitochondria and protects against renal tubular injury induced by albumin overload

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Summary. Proteinuria (albuminuria) is an important cause of aggravating tubulointerstitial injury. Previous studies have shown that autophagy activation can alleviate renal tubular epithelial cell injury caused by urinary protein, but the mechanism is not clear. Here, we investigated the role of clearance of damaged mitochondria in this protective effect. We found that albumin overload induces a significant increase in turnover of LC3-II and decrease in p62 protein level in renal proximal tubular (HK-2) cells *in vitro*. Albumin overload also induces an increase in mitochondrial damage. ALC, a mitochondrial torpent, alleviates mitochondrial damage induced by albumin overload and also decreases autophagy, while mitochondrial damage revulsant CCCP further increases autophagy. Furthermore, pretreatment of HK-2 cells with rapamycin reduced the amount of damaged mitochondria and the level of apoptosis induced by albumin overload. In contrast, blocking autophagy with chloroquine exerted an opposite effect. Taken together, our results indicated autophagy activation promotes removal of damaged mitochondria and protects against renal tubular injury caused by albumin overload. This further confirms previous research that autophagy activation is an adaptive response in renal tubular epithelial cells after urinary protein overload.

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Introduction

Proteinuria is an important feature of chronic diseases such as diabetic nephropathy (Erman et al., 2016), hypertensive renal damage (Lambers Heerspink and Gansevoort, 2015). Urinary protein is considered not only as a biomarker of glomerular injury; it can also be harmful to renal tubules, which could contribute to the progression of kidney diseases (Hodgkins and Schnaper, 2012). Albumin accounts for the majority of the protein in nephrotic urine (Erkan, 2013). A body of evidence indicates that albumin overload can lead to cell apoptosis (Sanchez-Nino et al., 2015), mitochondrial damage (Erkan et al., 2007), interstitial fibrosis (Nangaku, 2004), and inflammatory response (Morigi et al., 2002). Previous studies have shown that autophagic activation could alleviate renal tubular epithelial cell injury after urinary protein exposure, which is an adaptive response in renal tubular epithelial cell after urinary protein overload (Liu et al., 2014), although the involved mechanisms are poorly understood.

Mitochondria are essential organelles for eukaryotic cells and carry out diverse functions such as producing ATP and many biosynthetic intermediates (Wai and Langer, 2016). Mitochondrial damage can lead to a decrease in the activity of various antioxidant enzymes in the cytoplasm and a marked increase in reactive oxygen species, eventually resulting in cell injury (Murphy, 2016). Mitochondrial dysfunction has gained

recognition as a contributing factor in many diseases. The proximal tubules are particularly vulnerable to mitochondrial toxicity as they depend mainly on aerobic metabolism for energy supply (Hall et al., 2009). As such, mitochondrial dysfunction in the kidney plays a critical role in the pathogenesis of kidney diseases (Che et al., 2014). More importantly, *in vitro* evidence indicates that mitochondrial abnormalities have been observed in albumin-treated proximal tubule cells accompanied by mitochondrial cytochrome c release and impaired mitochondrial membrane potential (Zhuang et al., 2015), which was one of the main reasons for renal tubular epithelial cell apoptosis induced by excess albumin.

Autophagy is a regulated cellular self-eating process under different kinds of cellular stress and maintaining tissue homeostasis by elimination of damaged organelle and aggregated proteins (Mizushima, 2007). Moderate autophagy can remove aging and abnormal proteins, damaged mitochondria and other damaged organelles, which is beneficial for maintaining cell survival (Hartleben et al., 2014). Recent evidence demonstrates that autophagy alleviated cisplatin-induced renal tubular epithelial cell injury by ameliorating mitochondrial function (Zhao et al., 2017). Thus, we hypothesize that autophagy activation protects renal tubular epithelial cells from albumin induced injury by clearance of damaged mitochondria.

Therefore, in the present study we tested the effect of albuminuria-induced autophagy on mitochondrial dysfunction in HK-2 cells to explore the molecular mechanisms underlying tubular epithelial cell autophagy by albumin.

Materials and methods

Reagents and antibodies

Human proximal tubular epithelial HK-2 cell line was provided by China Center for Type Culture Collection (Wuhan, China). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium/F12 (DMEM/F12) were purchased from Gibco (Grand Island, NY, USA). Fatty-acid-free, low-endotoxin human serum albumin (HAS/ALB) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The mitochondrial torpent acetyl-l-carnitine (ALC), the mitochondrial damage revulsant carbonyl cyanide 3-chloro-phenylhydrazone (CCCP), the inducer of autophagy rapamycin (RAP) and the inhibitor of autophagy chloroquine (CQ) were purchased from Selleck Chemicals (Houston, TX, USA). JC-1 assay kit was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Mitochondria Isolation Kit was purchased from Applygen Technologies Inc. (Beijing, China). TUNEL apoptosis assay kit was obtained from Zoman Biotech. Co., Ltd. (Beijing, China). Methyl thiazolyltetrazolium (MTT) solution was purchased from Merck (Darmstadt, Germany). The GAPDH antibody was obtained from Cell Signaling Technology (Danvers, MA, USA). Light chain (LC) 3B, p62, and Cyt.c antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

Cell culture and treatment

Human proximal tubular epithelial HK-2 cells were cultured according to the procedure described in previous study (Liu et al., 2014). Briefly, HK-2 cells were cultured using DMEM/F12 medium containing 10% fetal calf serum, as well as penicillin and streptomycin, in a 37°C, 5% CO_2 incubator. When the culture confluence reached over 80%, it was digested with 0.25% trypsin and 0.05% ethylene diamine tetraacetic acid (EDTA). The culture medium was changed to serum-free medium 24 h before incubation with 8mg/ml albumin at 80% confluence. We used albumin concentration (8 mg/mL) that was based on a previous study, in which only a high dose of albumin at 8 mg/ml induced a significant autophagy in HK-2 cells but not in 0.1, 0.25, 0.5, 1, 2, 4 mg/ml by immuno-fluorescent assay and western blot assay (Liu et al., 2014). Treatments of cells in different experiment groups were described in results.

Western blotting

Total proteins were extracted in accordance with the instructions of the protein extraction kit (Promega, USA). Protein concentrations were measured using a BCA assay kit (Thermo, USA). Samples of 20 μg of protein were separated on 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred onto a 0.22 μm polyvinylidene difluoride (PVDF) membrane. The membranes were blocked with 5% non-fat milk for two hours on a shaker at room temperature, followed by incubation with appropriate dilutions of the primary specific antibody: rabbit anti-LC3B antibody (1:1000, Cell Signaling Technology, USA); rabbit anti-p62 antibody (1:1000, Cell Signaling Technology, USA); and rabbit anti-GAPDH antibody (1:1000, Cell Signaling Technology, USA) in TBS-T at 4°C overnight. The membranes were washed with TBS-T on the shaker. The secondary antibody was horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG (1:1000, Beyotime Biotechnology, China) incubation, which was carried out on the shaker at room temperature for one hour. Chemiluminescence was imaged in a Molecular Imager Chemi Doc™ XRS + WB with Image Lab™ software (Bio-Rad, CA, USA), and band intensity was measured by ImageJ software. All experiments were performed at least three times independently.

For determination of cytosolic cytochrome-c, Mitochondria Isolation Kit (Applygen, Beijing, China) was used for isolation of mitochondrial and cytosolic fractions. In brief, HK-2 cells were harvested and rinsed with ice-cold PBS. Then after several times of centrifugalization, cytosolic fraction was separated from

mitochondrial fraction. Western blot analysis of cytosolic cytochrome-c was performed as described previously.

JC-1 staining

The mitochondrial membrane potential was detected by JC-1 assay kit (Beyotime, China), as previously described (Acton et al., 2004). Briefly, Cells cultured in six-well plates after indicated treatments were incubated with an equal volume of JC-1 staining solution (5 μg/ml) at 37°C for 20 min and rinsed twice with PBS. Mitochondrial membrane potentials were monitored by determining the relative amounts of dual emissions from mitochondrial JC-1 monomers or aggregates using Zeiss Axio observer.D1 fluorescent microscope under Argon-ion 488 nm laser excitation. Mitochondrial depolarization is indicated by an increase in the green/red fluorescence intensity ratio.

Immunofluorescence staining

After incubation according to the research design, The cells were washed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde for 15 min. Then, they were permeabilized with 0.1% Triton X-100 for 10 min and blocked with BSA buffer for 30 min at room temperature. Subsequently, cells were incubated with anti-light chain 3B (LC3B) antibody (1:50, Cell Signaling Technology, USA) at 4°C overnight. After being rinsed with PBS, the cells were incubated for 1 h at room temperature with an appropriate secondary antibody. Finally, 4,6-diamidino-2-phenylindole (DAPI) (Beyotime, China) was used as a counterstain. Images were taken under OLYMPUS microscope (cellSens system) and OLYMPUS confocal microscope (FV 1200). The total number of HK-2 cells were counted in individual views. LC3-II dots were counted in individual HK-2 cell and the average of dots in at least 30 cells are presented in the figures.

TUNEL staining

The apoptosis was detected by TUNEL assay kit. TUNEL staining for apoptotic HK-2 cell was performed according to instructions of the manufacturer (Roche, CH). Briefly, HK-2 cells were planted on coverslips in six-well plates. After treatment, slides were washed three times with ice-cold PBS and fixed with 4% paraformaldehyde. The cells were incubated with 1% Triton X-100 for 5 min and then incubated at 37°C with 50 μl/slide TUNEL reaction mixture in darkness for 60 min. After incubation, the slides were washed three times and stained with Hoechst33258 for 5 min. The slides were examined using Zeiss Axio observer.D1 fluorescent microscope. The data expressed as the number of TUNEL-positive cells/the total number of cells in at least five high-power fields (× 400).

Cell viability assay

Cells were incubated with 5 mg/ml methyl thiazolyltetrazolium (MTT) solution for 4 h at 37°C. The formazan crystals were dissolved in dimethylsulfoxide. Optical density was determined at 570 nm with a plate reader (Thermo Labsystems, Multiskan MK3).

Statistical analysis

All statistical tests were performed with SPSS 16.0 (SPSS Inc., USA). All data were expressed as mean ± standard deviation (mean±SD). The significance of variables between groups were tested by one-way ANOVA. P value was considered as statistically significant if it is less than 0.05.

Results

Effect of albumin overload on autophagy in renal proximal tubular (HK-2) cells

In this study, we first utilized immunofluorescent technology to detect the expression of microtubule-associated protein 1 light chain 3 (LC3)-II, a key marker of autophagy, in HK-2 cells. As the Fig. 1A displays, HK-2 cells exposed to 8mg/ml albumin for 8h exhibited significantly more LC3-II positive dots than in untreated control cells. In parallel, significantly increased protein levels of LC3-II and reduced p62 levels were observed by western blot assay when cells were treated with 8 mg/ml albumin compared with control cells (Fig. 1B,C). These data indicate albumin is a major factor in triggering autophagic response in cultured HK-2 cells.

Effect of albumin overload on mitochondrial function in renal proximal tubular (HK-2) cells

Albumin exposure has been proved to cause mitochondrial injury in a previous study (Erkan et al., 2007). In our study, to evaluate the effects of albumin on mitochondrial function, we examined several independent parameters, including alterations in mitochondrial membrane potential and the release of cytochrome-c. Cytochrome-c, a useful marker of mitochondrial damage, resides in the mitochondrial membrane under normal conditions. With induction of apoptosis and alterations in the mitochondrial membrane, cytochrome-c translocates from the mitochondrial membrane to the cytoplasm (Eleftheriadis et al., 2016). As expected, a loss of mitochondrial membrane potential was seen in HK-2 cells, which was assessed by JC-1 staining (Fig. 2A). Consistently, western blot assays also showed a significant upregulation of cytosolic cytochrome-c with exposure of HK-2 cells to albumin (Fig. 2B), suggesting translocation of cytochrome-c from the mitochondrial membrane to the cytoplasm. The results revealed that

albumin overload induced mitochondrial abnormalities in HK-2 cells, which was consistent with previous studies (Erkan et al., 2007).

Mitochondrial injury is involved in albumin-induced autophagy activation

It was reported that mitochondrial dysfunction played an important role in autophagy activation (Zhao et al., 2016). To rule out the possibility that albumin-induced autophagy activation is mediated through mitochondrial injury in HK-2 cells, we used 2 drugs, including carbonyl cyanide 3-chlorophenylhydrazone (CCCP), mitochondrial damage revulsant (Kazlauskaite et al., 2014), and acetyl-l-carnitine (ALC), a mitochondrial torpent (Shen et al., 2008). As shown in Fig. 3A-C, the loss of mitochondrial membrane potential and cytochrome-c release were enhanced by CCCP application, while such effects were attenuated by ALC treatment. These data suggest that CCCP and ALC are functional in modulating mitochondrial function induced by albumin exposure.

We then tested if addition of CCCP or ALC affected albumin-induced autophagy in HK-2 cells. We observed

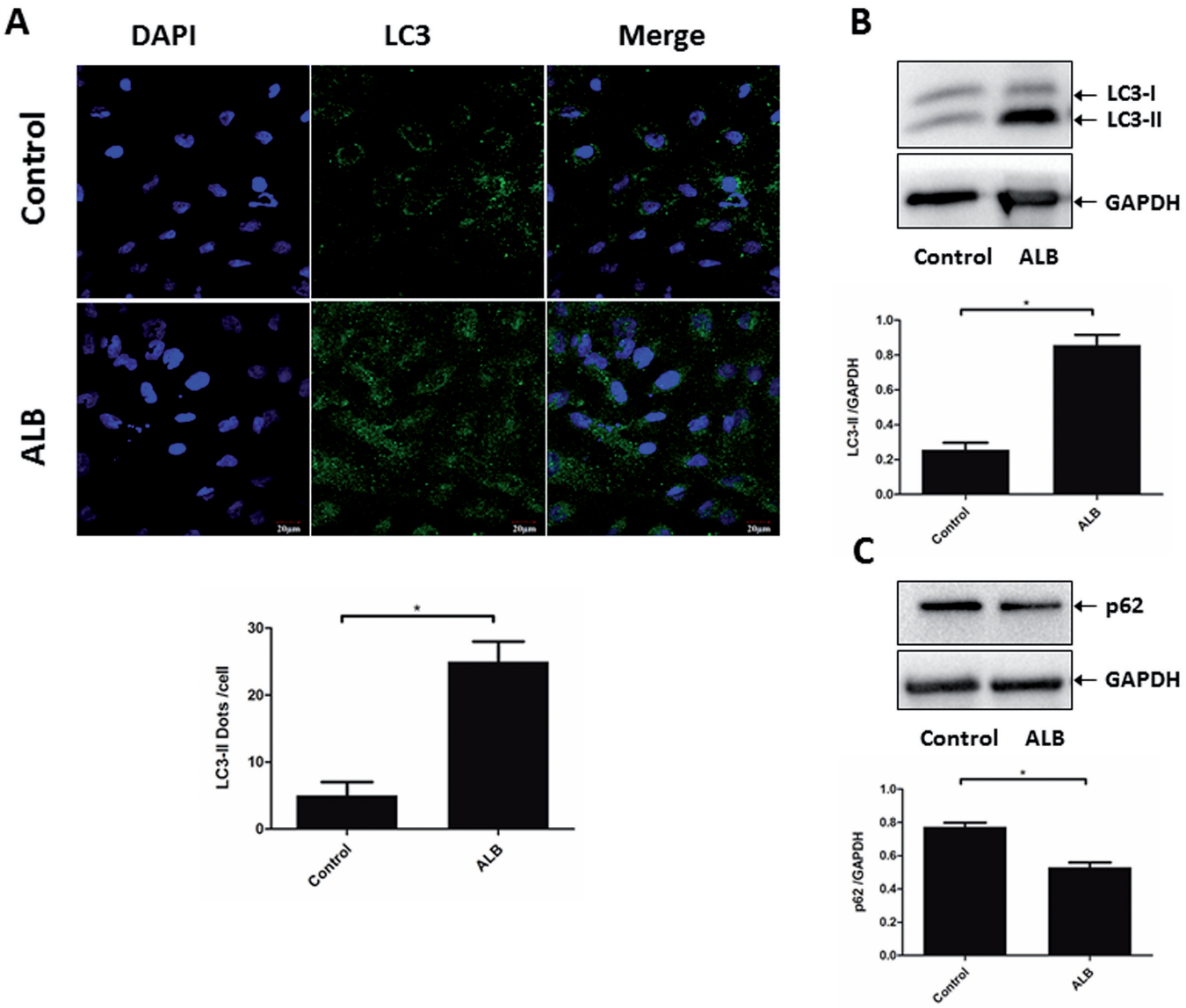

Fig. 1. Changes in autophagy response in HK-2 cells after exposure to albumin overload. **A.** Immunofluorescence staining and quantitative change of LC3-II (green) in HK-2 cells after exposure to 8mg/h albumin (ALB) for 8h. The nucleus was counter-stained by DAPI (blue). **B, C.** Western blot analysis of LC3 or p62 level in HK-2 cells after exposure to 8mg/h albumin (ALB) for 8h. Densitometry was performed for quantification and the ratio of LC3-II or p62 to GAPDH. *P<0.05, **P<0.01. Scale bars: 20 μm.

that compared with albumin treated alone, addition of CCCP to HK-2 cells significantly increased protein levels of LC3-II and reduced p62 levels as shown by Western blot analysis, while addition of ALC to HK-2 cells played an opposite role on the expression of LC3-II and p62 (Fig. 3D). Taken together, these results reveal that mitochondrial injury is a potential mechanism for triggering autophagy response by albumin.

Autophagy activation promotes removal of damaged mitochondria and protects against renal tubular injury induced by albumin overload

It has been demonstrated that autophagy response plays a preventive role in TEC injury induced by urinary protein overload (Liu et al., 2014). To further explore the potential mechanism, in comparison with albumin treatment, pretreatment of HK-2 cells with rapamycin, an autophagy inducer (Mizushima et al., 2010), exhibited marked enhancement of autophagic activities as assessed by increased LC3-II protein levels and decreased p62 levels, as well as alleviating mitochondrial injury as assessed by the recovery of mitochondrial membrane potential and reduced cytochrome-c release. In contrast, blocking autophagy with chloroquine exerted an opposite effect (Fig. 4A-D).

Finally, we demonstrated the critical role of autophagy on HK-2 cells survival after exposure to albumin. As revealed by TUNEL assay and MTT assay, addition of rapamycin significantly reduced cell apoptosis and increased cell viability. In contrast, chloroquine treatment increased cell apoptosis and increased cell viability (Fig. 5A-C). These experiments clearly showed that autophagy plays a preventive role in

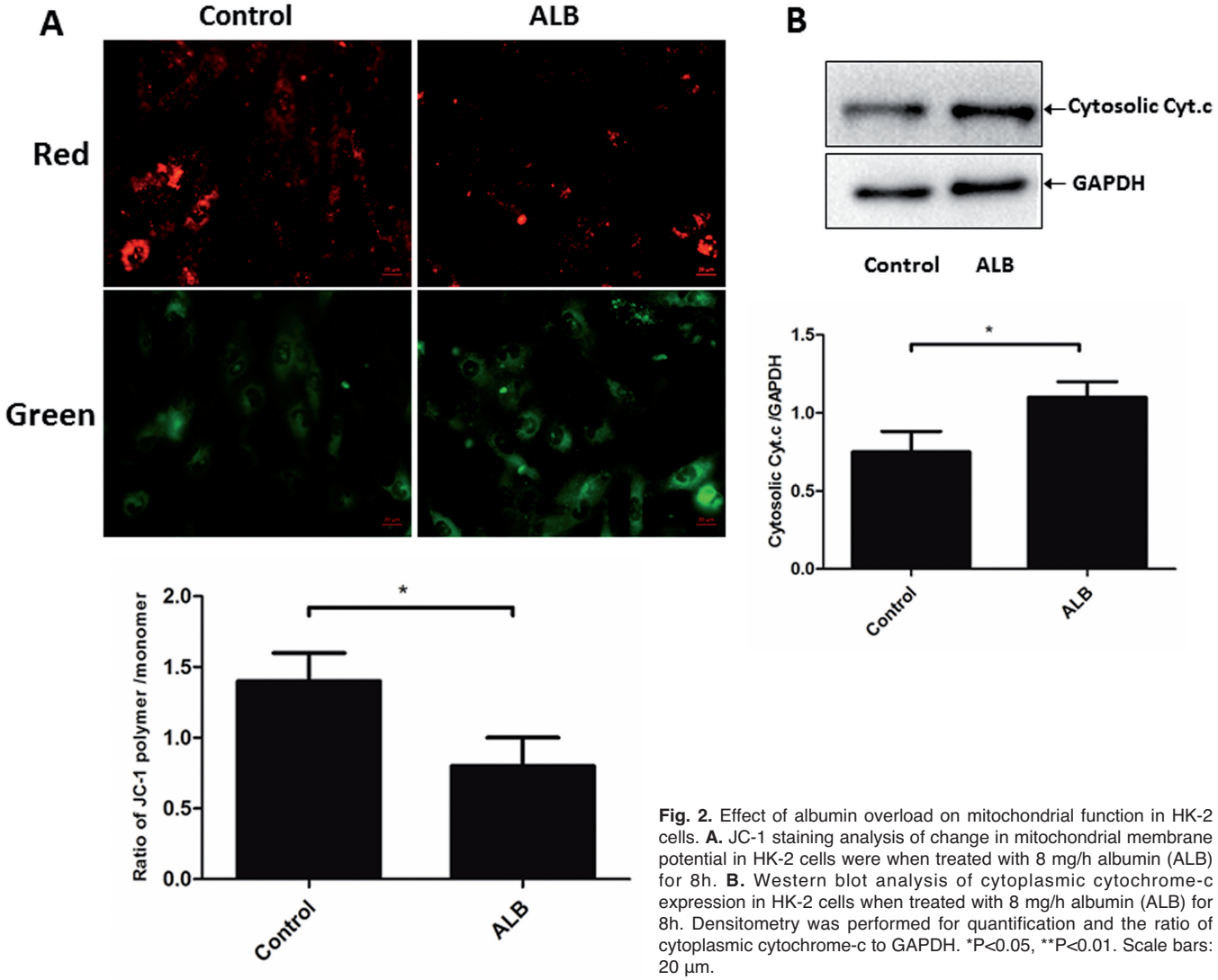

Fig. 2. Effect of albumin overload on mitochondrial function in HK-2 cells. **A.** JC-1 staining analysis of change in mitochondrial membrane potential in HK-2 cells were when treated with 8 mg/h albumin (ALB) for 8h. **B.** Western blot analysis of cytoplasmic cytochrome-c expression in HK-2 cells when treated with 8 mg/h albumin (ALB) for 8h. Densitometry was performed for quantification and the ratio of cytoplasmic cytochrome-c to GAPDH. *P<0.05, **P<0.01. Scale bars: 20 μm.

HK-2 cell injury induced by albumin overload by promoting removal of damaged mitochondria.

Discussion

Previous studies have indicated that autophagy activation in response to urinary protein exposure represents a protective response, which alleviates TEC injury. In the present study, we investigated the role of clearance of damaged mitochondria in this protective effect. We found that autophagy activation promotes removal of damaged mitochondria and protects against renal tubular injury caused by albumin overload.

The human body usually excretes 40 to 80 mg of proteins through urine per day, from which 30-40% are albumin (Gorriz and Martinez-Castelao, 2012). As the most prominent protein in nephrotic urine, urinary albumin has been proved to be toxic for renal tubular epithelial cells (Li et al., 2010). In this study, we investigated the effect of 8 mg/ml albumin on autophagy in renal tubular epithelial cells on the basis of a previous study. The result showed that HK-2 cells treated with 8mg/ml albumin for 8 h can also induce autophagy, suggesting that albumin may be the major component of urinary proteins in mediating autophagy activation in TECs. Our result is consistent with the study by Allouchand Munusamy (2017). However, it is worth noting that albumin has a contrary effect on autophagy. In another study, Nolin et al. found excess albumin exposure caused dysfunctional autophagy in

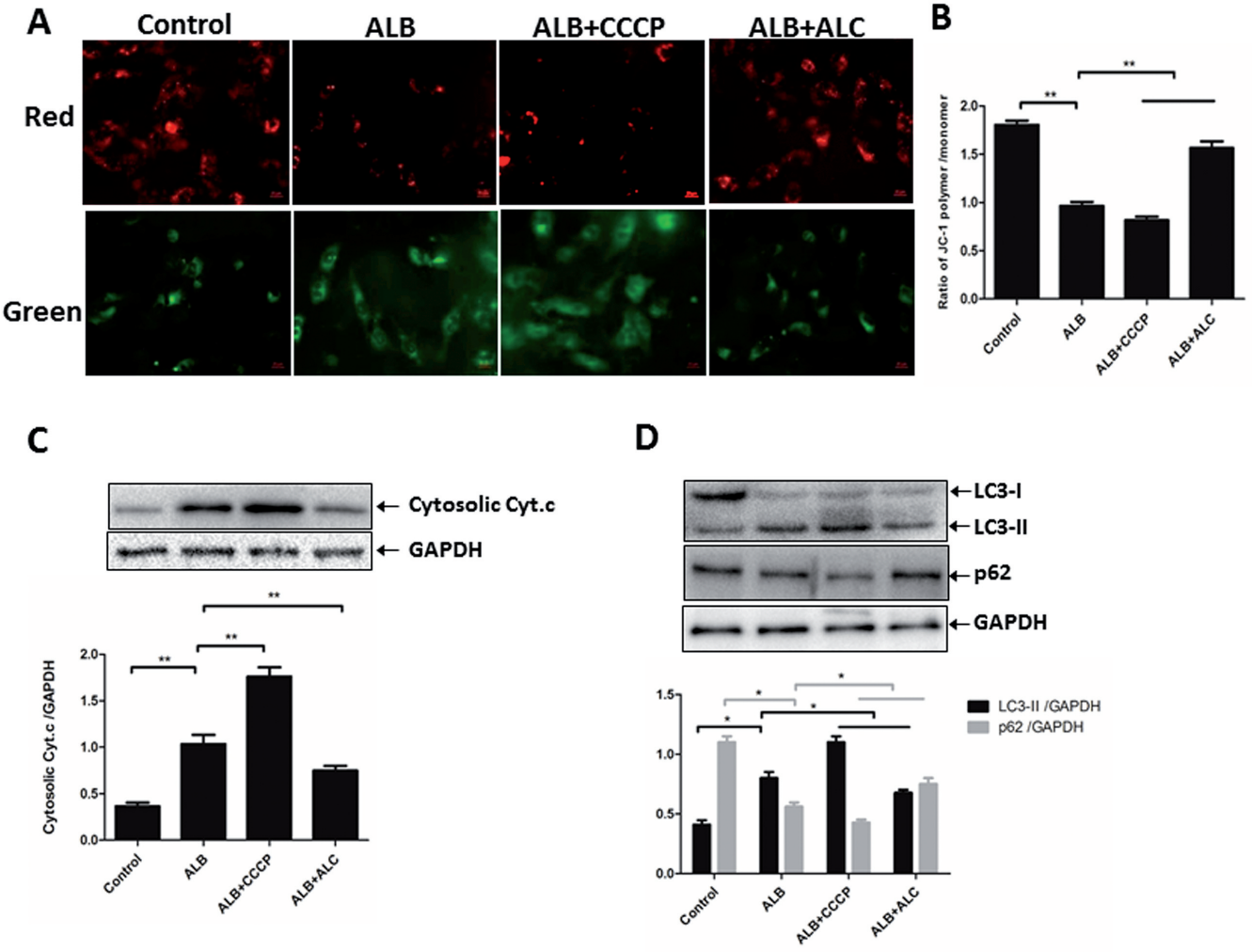

Fig. 3. Effects of CCCP or ALC on mitochondrial function and autophagic activity in HK-2 cells after exposure to albumin overload. **A, B.** JC-1 staining analysis of change in mitochondrial membrane potential in HK-2 cells after exposure to albumin (ALB, 8 mg/ml), with carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 10 μM) or acetyl-l-carnitine (ALC, 10 μM) for 8 h. **C.** Western blot analysis of cytoplasmic cytochrome-c expression in HK-2 cells treated as described in **A**. Densitometry was performed for quantification and the ratio of cytoplasmic cytochrome-c to GAPDH. **D.** Western blot analysis of LC3 or p62 level in HK-2 cells treated as described in **A**. Densitometry was performed for quantification and the ratio of LC3-II or p62 to GAPDH. *P<0.05, **P<0.01. Scale bars: 20 μm.

the proximal tubule treated for 5 days (Nolin et al., 2016). The major reason for the differences could be due to the duration of the experimental insult on autophagy.

The effect of albumin overload on mitochondrial damage in renal tubular epithelial cells has been discovered previously (Zhuang et al., 2015). It has been recognized that mitochondria are the major targets in albumin-induced apoptosis in proximal tubule cells (Erkan et al., 2007). To investigate the role of the mitochondria dysfunction in albumin-induced renal tubular autophagy, we found that the enhanced autophagic activities as assessed by increased LC3-II protein expression and decreased p62 levels in TECs treated with albumin were obviously attenuated by ALC addition, a protective nutrient of mitochondria, and further enhanced by mitochondrial uncoupling agent CCCP. Based on our experimental results, it is conceivable to propose that albumin-activated autophagy might be mediated, at least to some extent, through a mitochondrial injury. The effects of damaged mitochondria on autophagy levels were also present in other pathological conditions, for instance, disruption of renal tubular mitochondrial quality control by Myo-inositol oxygenase in diabetic kidney disease (Zhan et al., 2015).

Whether increased autophagy promotes clearance of damaged mitochondria in TECs has not been studied. In our study, we demonstrated that mitochondrial injury caused by albumin, as assessed by cytochrome c release and loss of mitochondrial membrane potential, were attenuated by autophagy enhancer rapamycin addition

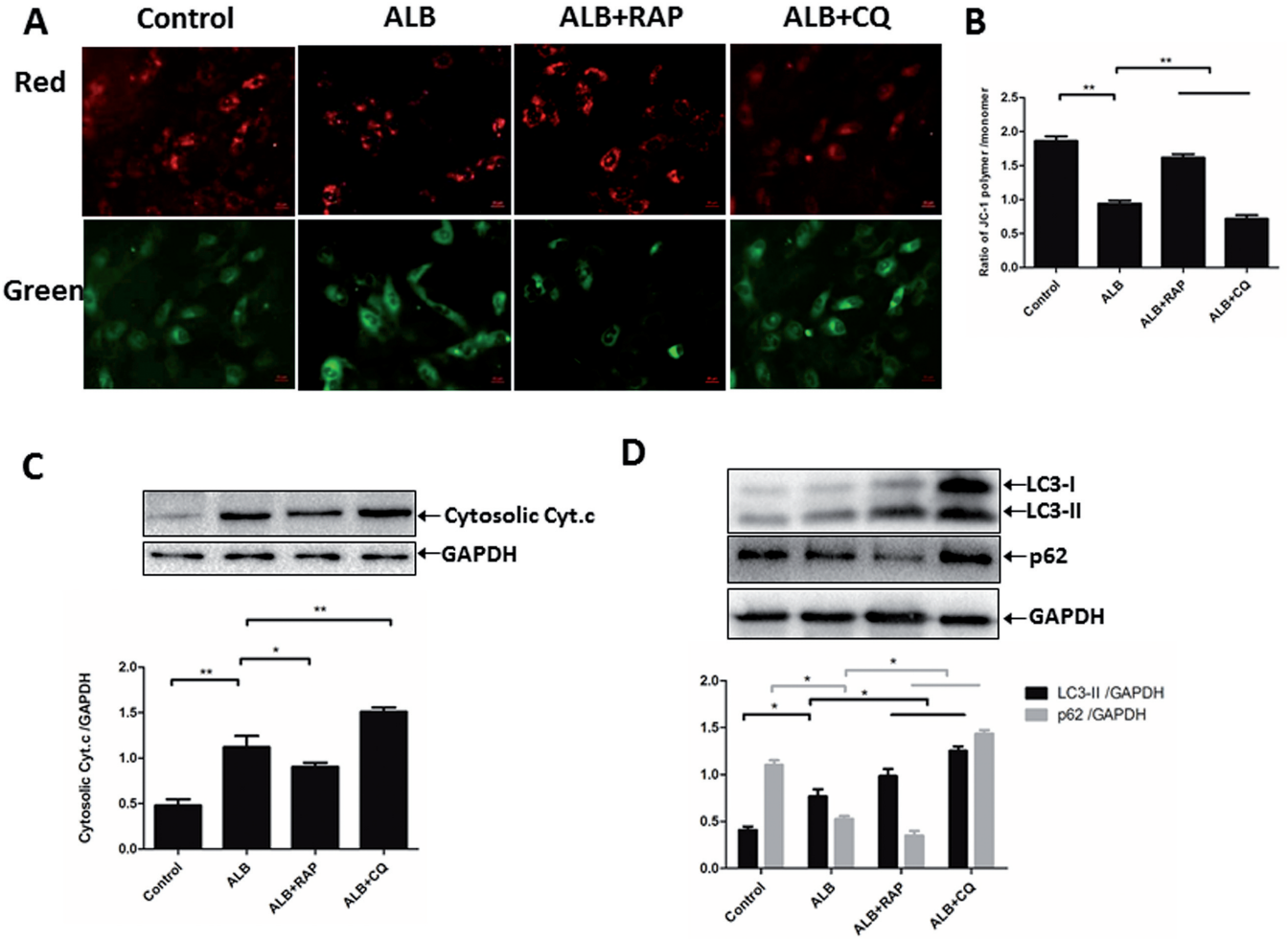

Fig. 4. Effects of rapamycin or chloroquine on mitochondrial function and autophagic activity in HK-2 cells after exposure to albumin overload. **A, B.** JC-1 staining analysis of change in mitochondrial membrane potential in HK-2 cells after exposure to albumin (ALB, 8 mg/ml), with rapamycin (RAP, 10 μM) or chloroquine (CQ, 10 μM) for 8 h. **C.** Western blot analysis of cytoplasmic cytochrome-c expression in HK-2 cells treated as described in **A**. Densitometry was performed for quantification and the ratio of cytoplasmic cytochrome-c to GAPDH. **D.** Western blot analysis of LC3 or p62 level in HK-2 cells treated as described in **A**. Densitometry was performed for quantification and the ratio ofLC3-II or p62 to GAPDH. *P<0.05, **P<0.01. Scale bars: 20 μm.

but enhanced by autophagy inhibitor chloroquine. Therefore, our results suggest autophagy can mitigate mitochondrial damage by scavenging damaged mitochondria, an explanation for preventing renal tubular epithelial cells from injury induced by excessive urinary albumin. In recent years, a growing body of

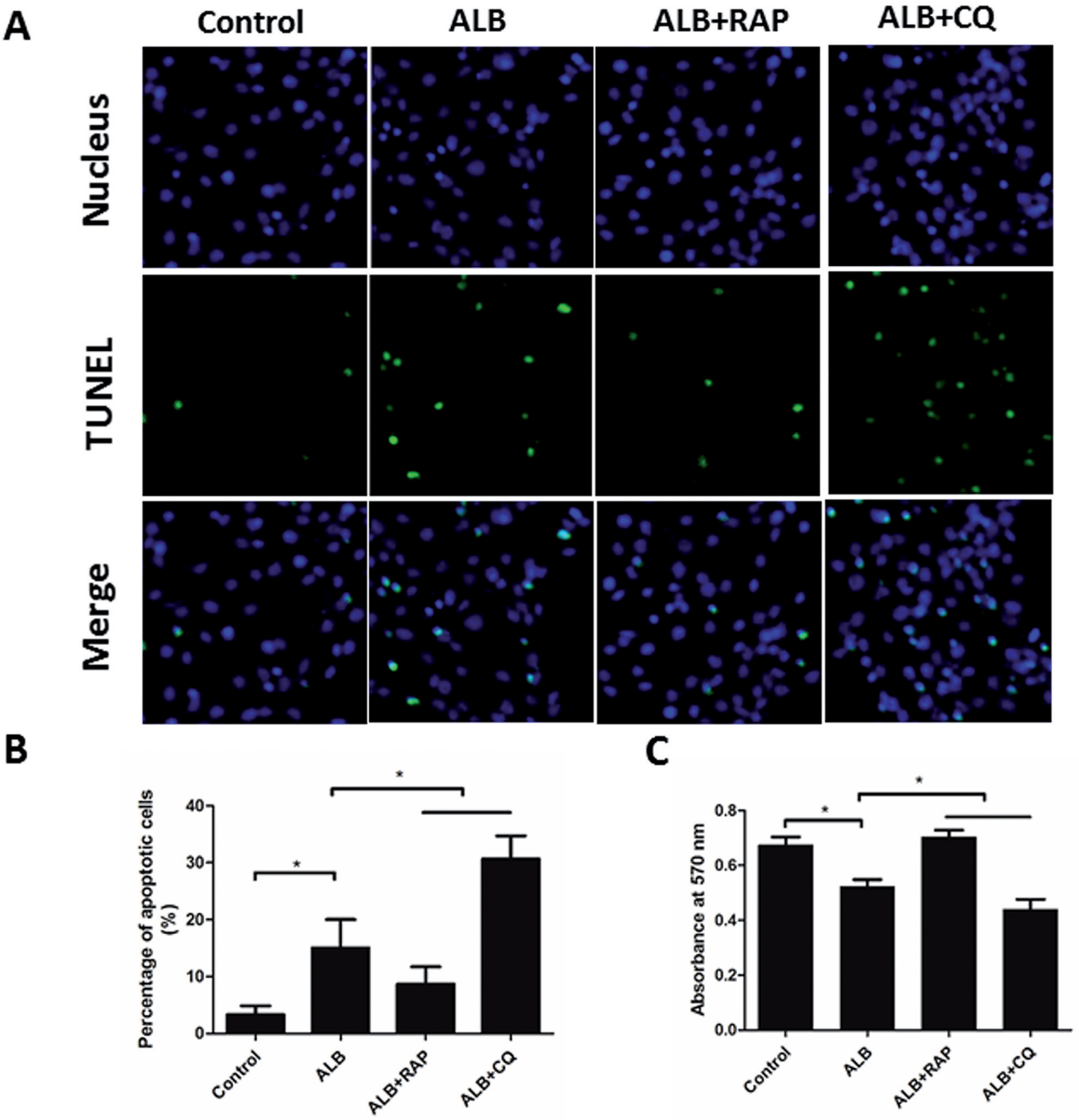

Fig. 5. Effects of rapamycin or chloroquine on HK-2 cell injury and viability induced by albumin overload. **A, B.** Apoptosis was assessed by TUNEL in HK2 cells after exposure to albumin (ALB, 8 mg/ml), ALB (8 mg/ml) plus rapamycin (RAP, 10 μM) or ALB (8 mg/ml) plus chloroquine (CQ, 10 μM) for 8 h. **C.** Cell viability was assessed by MTT assay as described in **A**.*P<0.05 and **P<0.01. × 400.

research demonstrates that mitophagy, the selective elimination of impaired mitochondria via autophagy, is an important mechanism of mitochondrial quality control in physiological and pathological conditions (Williams and Ding, 2015). However, whether albumin-triggered autophagy in renal tubular epithelial cells is mitophagy has not been studied in our experiment, further research is needed to examine this problem.

Removal of damaged mitochondria by autophagy may also be deleterious rather than protective. As such, mitochondrial autophagy promoted cellular injury in nephropathic cystinosis (Sansanwal et al., 2010). Our results demonstrate that rapamycin alleviated TEC apoptosis and improved cell viability, while chloroquine had the opposite effect, which indicates that autophagy activation plays a key role in removing damaged mitochondria and protecting against renal tubular injury under albumin overload.

Overall, autophagy plays a vital role in regulating renal tubular epithelial cells survival under excessive albumin (Fig. 6). On one hand, when TECs are in relative deficiency of autophagy, cell apoptosis would occur due to mitochondrial dysfunction caused by albumin overload. On the other hand, autophagy enhanced by rapamycin would mitigate mitochondrial injury through eliminating damaged mitochondria, ultimately improving cell survival, which mounts an adaptive response in TECs after albumin overload.

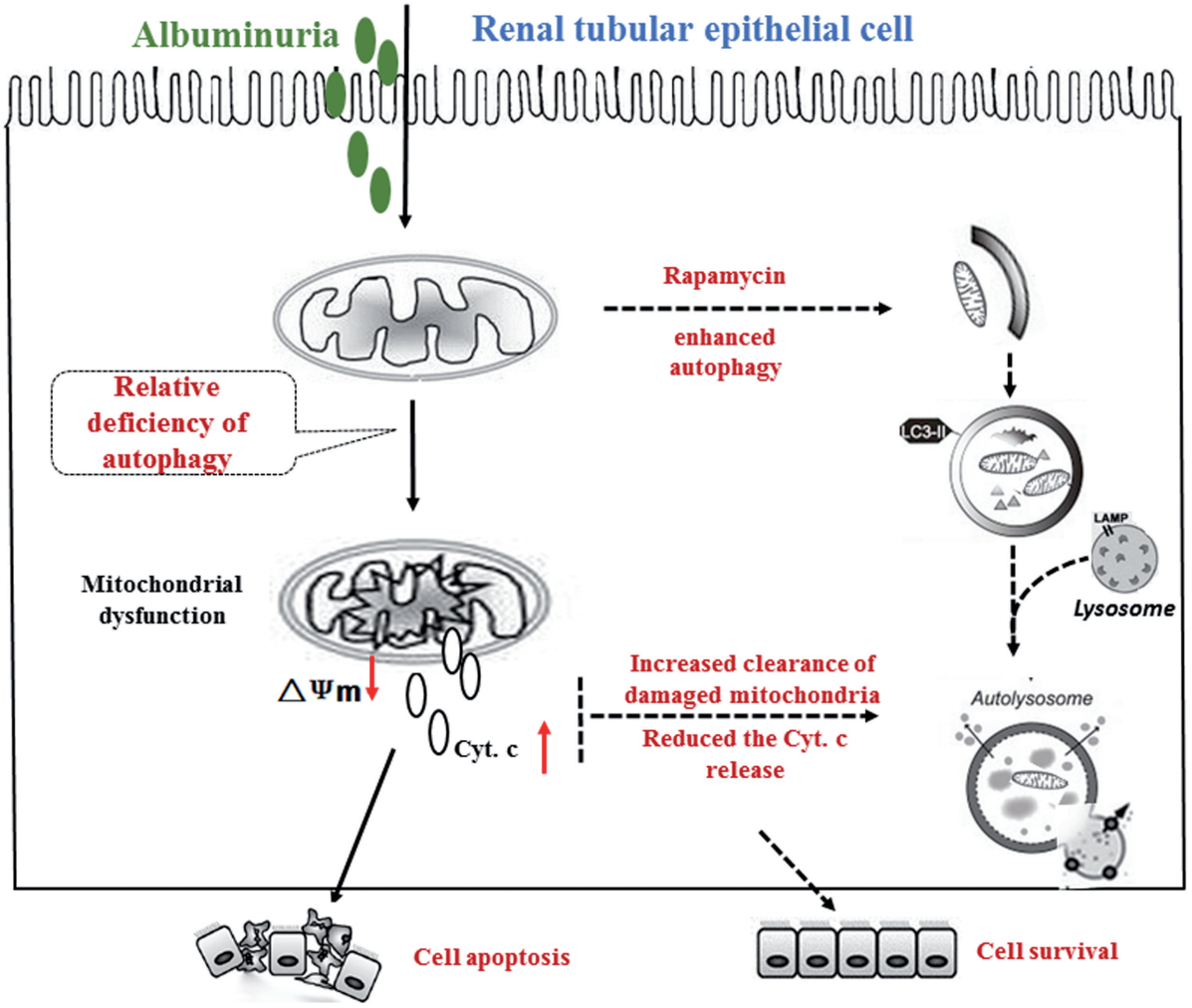

Fig. 6. The mechanism chart of autophagy promoting cell survival through removal of damaged mitochondria in renal tubular epithelial cells after urinary albumin overload. When cells are relative deficient in autophagy, albumin overload would result in mitochondrial injury, including impaired mitochondrial membrane potential and release of cytochrome c from mitochondria to cytoplasm, eventually leading to cell apoptosis, depicted in the solid line. However, when cells are treated with rapamycin to enhance autophagy, autophagy activation would promote clearance of damaged mitochondria to improve cell survival after urinary albumin overload, depicted in the dotted line.

Conclusion

In summary, our results indicated autophagy activation promotes removal of damaged mitochondria and protects against renal tubular injury caused by albumin overload. This further confirms previous research that autophagy activation is an adaptive response in renal tubular epithelial cells after urinary protein overload, and promoting autophagic flux in TECs might be beneficial in clinical management of nephropathies with refractory proteinuria.

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Conflict of interest. The authors declare no conflict of interest.
Authors' Contributions. Jin Tan and Miaohong Wang contributed equally to this work.

References

Acton B.M., Jurisicova A., Jurisica I. and Casper R.F. (2004). Alterations in mitochondrial membrane potential during preimplantation stages of mouse and human embryo development. Mol. Human Reprod. 10, 23-32.

Allouch S. and Munusamy S. (2017). Metformin attenuates albumin-induced alterations in renal tubular cells *in vitro*. J. Cell Physiol. 232, 3652-3663.

Che R., Yuan Y., Huang S. and Zhang A. (2014). Mitochondrial dysfunction in the pathophysiology of renal diseases. American journal of physiology. Renal Physiol. 306, F367-378.

Eleftheriadis T., Pissas G., Liakopoulos V. and Stefanidis I. (2016). Cytochrome c as a potentially clinical useful marker of mitochondrial and cellular damage. Front. Immunol. 7, 279.

Erkan E. (2013). Proteinuria and progression of glomerular diseases. Pediat. Nephrol. 28, 1049-1058.

Erkan E., Devarajan P. and Schwartz G.J. (2007). Mitochondria are the major targets in albumin-induced apoptosis in proximal tubule cells. J. Am. Soc. Nephrol. 18, 1199-1208.

Erman O., Erman A., Vodonos A., Gafter U. and van Dijk D.J. (2016). A new cutoff for abnormal proteinuria in diabetes mellitus patients: Relationship to albuminuria. Isr. Med. Assoc. J. 18, 418-421.

Gorriz J.L. and Martinez-Castelao A. (2012). Proteinuria: Detection and role in native renal disease progression. Transplant. Rev. 26, 3-13.

Hall A.M., Unwin R.J., Parker N. and Duchen M.R. (2009). Multiphoton imaging reveals differences in mitochondrial function between nephron segments. J. Am. Soc. Nephrol. 20, 1293-1302.

Hartleben B., Wanner N. and Huber T.B. (2014). Autophagy in glomerular health and disease. Semin. Nephrol. 34, 42-52.

Hodgkins K.S. and Schnaper H.W. (2012). Tubulointerstitial injury and the progression of chronic kidney disease. Pediat. Nephrol. 27, 901-909.

Kazlauskaite A., Kondapalli C., Gourlay R., Campbell D.G., Ritorto M.S., Hofmann K., Alessi D.R., Knebel A., Trost M. and Muqit M.M. (2014). Parkin is activated by pink1-dependent phosphorylation of ubiquitin at ser65. Biochem. J. 460, 127-139.

Lambers Heerspink H.J. and Gansevoort R.T. (2015). Albuminuria is an appropriate therapeutic target in patients with ckd: The pro view. Clin. J. Am. Soc. Nephrol. 10, 1079-1088.

Li X., Pabla N., Wei Q., Dong G., Messing R.O., Wang C.Y. and Dong Z. (2010). Pkc-delta promotes renal tubular cell apoptosis associated with proteinuria. J. Am. Soc. Nephrol. 21, 1115-1124.

Liu W.J., Luo M.N., Tan J., Chen W., Huang L.Z., Yang C., Pan Q., Li B. and Liu H.F. (2014). Autophagy activation reduces renal tubular injury induced by urinary proteins. Autophagy 10, 243-256.

Mizushima N. (2007). Autophagy: Process and function. Genes Dev. 21, 2861-2873.

Mizushima N., Yoshimori T. and Levine B. (2010). Methods in mammalian autophagy research. Cell 140, 313-326.

Morigi M., Macconi D., Zoja C., Donadelli R., Buelli S., Zanchi C., Ghilardi M. and Remuzzi G. (2002). Protein overload-induced NF-kappab activation in proximal tubular cells requires H(2)O(2) through a pkc-dependent pathway. J. Am. Soc. Nephrol. 13, 1179-1189.

Murphy M.P. (2016). Understanding and preventing mitochondrial oxidative damage. Biochem. Soc. Transact. 44, 1219-1226.

Nangaku M. (2004). Mechanisms of tubulointerstitial injury in the kidney: Final common pathways to end-stage renal failure. Intern. Med. 43, 9-17.

Nolin A.C., Mulhern R.M., Panchenko M.V., Pisarek-Horowitz A., Wang Z., Shirihai O., Borkan S.C. and Havasi A. (2016). Proteinuria causes dysfunctional autophagy in the proximal tubule. Am. J. Physiol. Renal Physiol. 311, F1271-F1279.

Sanchez-Nino M.D., Fernandez-Fernandez B., Perez-Gomez M.V., Poveda J., Sanz A.B., Cannata-Ortiz P., Ruiz-Ortega M., Egido J., Selgas R. and Ortiz A. (2015). Albumin-induced apoptosis of tubular cells is modulated by basp1. Cell Death Dis. 6, e1644.

Sansanwal P., Yen B., Gahl W.A., Ma Y., Ying L., Wong L.J. and Sarwal M.M. (2010). Mitochondrial autophagy promotes cellular injury in nephropathic cystinosis. J. Am. Soc. Nephrol. 21, 272-283.

Shen W., Liu K., Tian C., Yang L., Li X., Ren J., Packer L., Cotman C.W. and Liu J. (2008). R-alpha-lipoic acid and acetyl-l-carnitine complementarily promote mitochondrial biogenesis in murine 3t3-l1 adipocytes. Diabetologia 51, 165-174.

Wai T. and Langer T. (2016). Mitochondrial dynamics and metabolic regulation. Trends Endocrinol. Metab. TEM 27, 105-117.

Williams J.A. and Ding W.X. (2015). A mechanistic review of mitophagy and its role in protection against alcoholic liver disease. Biomolecules 5, 2619-2642.

Zhan M., Usman I.M., Sun L. and Kanwar Y.S. (2015). Disruption of renal tubular mitochondrial quality control by myo-inositol oxygenase in diabetic kidney disease. J. Am. Soc. Nephrol. 26, 1304-1321.

Zhao B., Qiang L., Joseph J., Kalyanaraman B., Viollet B. and He Y.Y. (2016). Mitochondrial dysfunction activates the ampk signaling and autophagy to promote cell survival. Genes Dis. 3, 82-87.

Zhao C., Chen Z., Xu X., An X., Duan S., Huang Z., Zhang C., Wu L., Zhang B., Zhang A., Xing C. and Yuan Y. (2017). Pink1/parkin-mediated mitophagy play a protective role in cisplatin induced renal tubular epithelial cells injury. Exp. Cell Res. 350, 390-397.

Zhuang Y., Yasinta M., Hu C., Zhao M., Ding G., Bai M., Yang L., Ni J., Wang R., Jia Z., Huang S. and Zhang A. (2015). Mitochondrial dysfunction confers albumin-induced nlrp3 inflammasome activation and renal tubular injury. American journal of physiology. Renal Physiol. 308, F857-866.

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