Am J Transl Res 2021;13(8):8754-8765
www.ajtr.org /ISSN:1943-8141/AJTR0129871

*Original Article*

# High uric acid promotes mitophagy through the ROS/CaMKIIδ/Parkin pathway in cardiomyocytes *in vitro* and *in vivo*

Kai Gao[1*], Yanbing Li[2*], Yiwan Su[3], Zhishan Lin[3], Xiangbin Yang[3], Meiling Xu[1], Yanting Huang[3], Shuqin Chen[3], Yang Xie[1], Zhi Li[3]

*[1]Emergency Department, The Second Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong Province, China; [2]Department of Cardiology, Beijing Youan Hospital, Capital Medical University, Beijing, China; [3]Department of Cardiology, The Second Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong Province, China. [*]Equal contributors and co-first authors.*

Received January 10, 2021; Accepted June 13, 2021; Epub August 15, 2021; Published August 30, 2021

**Abstract:** Background: Increasing evidence has suggested that high uric acid (HUA) is closely related to cardiovascular disease (CVD). Mitophagy abnormalities have been reported to participate in multiple pathogenic processes of CVD. However, the potential molecular mechanisms remain unclear. Herein, we investigated the effect of HUA-induced mitophagy and its potential molecular mechanism in cardiomyocytes. Methods: We established a model of cardiomyocytes induced by HUA *in vitro* and *in vivo*. Mitochondrial membrane potential (MMP), reactive oxygen species (ROS) production and adenosine triphosphate (ATP) content were measured. The mitophagy-related protein expression of LC3B-II, Parkin, $Ca^{2+}$/calmodulin-dependent protein kinase II δ (CaMKIIδ) and P62 was measured by Western blot. Based on the colocalization of lysosomes and mitochondria, a confocal microscope was used to detect mitophagy. Additionally, we established a mitophagy inhibitor group (3-MA) and CaMKIIδ inhibitor group (KN-93) to verify the pathway. Results: In the HUA stimulation model, ROS production was increased, and mitochondrial injury indexes (MMP and ATP contents) were decreased. Moreover, these indicators were reversed by 3-MA and KN-93. Under HUA stimulation, the expression of LC3B-II, Parkin, CaMKIIδ and P62 increased significantly. Furthermore, these protein levels were reduced by 3-MA and KN-93. Conclusion: HUA can promote cardiomyocyte mitophagy activation through the ROS/CaMKIIδ/parkin pathway axis. This study may provide a new target and theoretical basis for the prevention and treatment of HUA-related metabolic heart disease in the future.

**Keywords:** High uric acid, mitophagy, ROS, cardiomyocytes

## Introduction

Recent research shows that high uric acid (HUA) is closely related to cardiovascular disease (CVD) [1-6] and has been listed as the fourth highest cardiovascular risk factor by the Cardiology Journal [7]. The treatment of reducing uric acid has been shown to improve the survival prognosis of patients with adult CVD. However, the molecular mechanisms and significance are not clear.

HUA increases oxidative stress in cardiomyocytes. Our previous studies have unveiled increased reactive oxygen species (ROS) production in cardiomyocytes by HUA treatment [8, 9]. The increase in ROS is closely related to mitochondrial dysfunction [10]. Although the key role of HUA in mitochondrial dysfunction in CVD has not yet been determined, increasing evidence has shown that oxidative stress plays a vital role in CVD associated with mitochondrial dysfunction, and both ROS overproduction and mitochondrial dysfunction may be associated with CVD.

As a process of selectively degrading mitochondria, mitophagy plays a significant role in the quality control of mitochondria. Mitochondria play an important role not only in energy production but also as a major source of ROS. However, excessive ROS produced by mitochondrial damage can damage mitochondrial proteins and DNA, leading to more pronounced

mitochondrial dysfunction [11]. Therefore, rapid and selective clearance of damaged mitochondria by mitophagy is critical for cell survival. Two known pathways primarily regulate mitophagy, one of which is mediated by PTEN-induced putative kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin [12, 13]. Parkin is normally located in the cytoplasm, and once the mitochondrial membrane is damaged, it will rapidly translocate to the mitochondria, ubiquitinating the mitochondrial membrane protein [14] and thereby labeling the damaged mitochondria. The adaptor protein (p62) then binds to both ubiquitinated proteins on mitochondria and LC3B-II on autophagosomes. Increasing evidence suggests that mitophagy is involved in the pathogenesis of a variety of CVDs, including cardiomyocyte ischemia [15], cardiomyocyte infarction [16] and heart failure [17]. Under stress, cardiomyocyte mitophagy is enhanced, but whether HUA can affect mitophagy through oxidative stress and disruption of mitochondrial homeostasis has not been reported.

Additionally, $Ca^{2+}$ plays a vital regulatory role in the function and metabolic activities of cardiomyocytes. Homeostasis of [$Ca^{2+}$] regulation in cardiomyocytes is a prerequisite for maintaining normal function of the heart. The disorder of $Ca^{2+}$ regulation not only affects the contractile and diastolic function of cardiomyocytes but also causes $Ca^{2+}$ overload, which is the ultimate pathway for cell damage and is associated with a variety of CVDs, such as ischemic cardiomyopathy [18], cardiac hypertrophy [19], and heart failure [20]. $Ca^{2+}$/calmodulin-dependent protein kinase II δ (CaMKIIδ) is an important signaling molecule that regulates [$Ca^{2+}$]. Its increased expression and enhanced activity can induce cardiomyocyte apoptosis and cardiac hypertrophy, which plays an important role in ischemic cardiomyopathy and even heart failure. Other studies have shown that CaMKIIδ can regulate mitochondrial dynamics, while mitochondrial dynamics are related to mitophagy [23-25].

Our previous study showed that HUA affected cardiomyocytes through oxidative stress and other pathways [9], but it is still unknown whether HUA can affect cardiomyocytes through the mitophagy pathway. Parkin mediates mitophagy in general, but whether Parkin mediates HUA-induced mitophagy in HUA-induced injury is unknown. Delineation of how Parkin-mitophagy affects cardiomyocyte damage caused by HUA will enable the identification of new targets for the treatment of HUA-related metabolic heart disease. We speculate that oxidative stress and calcium overload induced by HUA in cardiomyocytes activate the CaMKIIδ pathway to induce mitophagy as a cytoprotective response.

## Materials and methods

### *Reagents*

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT, USA). Uric acid and 3-methyladenine (3-MA) were purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA). The following polyclonal primary antibodies were used in this study: anti-CaMKIIδ (Abcam, Cambridge, UK), anti-Parkin (Abcam, Cambridge, UK), anti-p62 (Abcam, Cambridge, UK), anti-GAPDH (Sigma-Aldrich Co.) and anti-LC3B (Sigma-Aldrich Co.). Collagenase and trypsin were purchased from Sigma-Aldrich Co.

### *Cardiomyocyte culture and HUA treatment in vitro*

The cardiomyocytes of newborn rats were prepared by the Sprague-Dawley (SD) rat myocardial enzymolysis method and cultured as described above [20] for 0, 6, 12, 24, and 48 h after exposure to UA. It was found that 24 h was the best processing time. Cells were also cultured at 0, 5, 10, 15, and 20 mg/dl after exposure to UA. Overall, 24 h and 15 mg/dl were the best processing time and concentration, respectively, and thus cells were stimulated with HUA (15 mg/dl) for 24 h and then used for subsequent experiments and analysis.

### *Detection of cardiomyocyte mitochondrial morphology*

Mitophagy is observed by the colocalization of lysosomes and mitochondria. H9C2 cells were cultivated with LysoTracker Red (50 nM) and MitoTracker Green (100 nM, molecular probe, Eugene, or USA) for 45 minutes. Confocal images were obtained via a Zeiss LSM 880 (Zeiss, Germany). ImageJ software was used to detect the number of MitoTracker- and LysoTracker-positive lesions.

*Determination of ROS levels*

Cardiomyocytes were subcultured in a 6-well plate ($2.0\times10^5$ cells/well) for 24 h and exposed to HUA (15 mg/dl) for 24 h. The cells were stained with 10 mM DCFH-DA at 37°C for 30 minutes, as described in [26]. Flow cytometry was used to detect stained cells with an excitation wavelength of 530 nm and an emission wavelength of 480 nm.

*Determination of MMP*

The JC-1 kit (Sigma-Aldrich) was used for MMP measurement. After stimulation, the cells were incubated with JC-1 staining solution at 37°C for 20 minutes and then washed twice with JC-1 staining buffer. Flow cytometry and fluorescence microscopy were used to detect MMP.

*Determination of ATP levels*

The ATP content in cardiomyocytes was determined by using an ATP kit (Beyotime). Briefly, cardiomyocytes were lysed with a cellular ATP release agent, and then the lysate was diluted in ATP detection solution and mixed with luciferase solution. A luminometer was used to measure bioluminescence. Besides, the ATP content was estimated according to the standard curve. The results were normalized to cellular protein concentrations.

*Animals*

The animal study was conducted in strict accordance with the recommendations in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The animal experiment ethics committee of Shantou University approved this animal experiment (License no: syxk2007-0097). Male 7-week-old SD male rats weighing 200-220 g were acquired from the Experimental Animal Center, Shantou University Medical College. All operations were performed under pentobarbital sodium anesthesia, and their pain was relieved. The rats were fed a standard diet, kept in a separate cage, had a regular light-dark cycle, and were allowed to acclimate to the lab for a week. Pentobarbital sodium (50 mg/kg intraperitoneally) was used to anesthetize the animals. Individual ventricular myocytes were harvested as previously described [27]. Male SD rats at the age of 8 weeks were randomly divided into 3 groups (4 rats in each group) for treatment: control, HUA and HUA+3-MA groups. For HUA treatment, after overnight fasting for 18 h, the rats were intraperitoneally injected with potassium oxonate (300 mg/kg) and given hypoxanthine (500 mg/kg) by gavage for 12 h to establish an acute hyperuricemia model. The volume of the drug was calculated from the weight measured immediately before each dose. Then, serum UA levels were measured by phosphotungstic acid at different times [28]. Left ventricular myocardial tissue was resected in the control, UA and HUA+3-MA rats. All tissue samples were stored in liquid nitrogen immediately.

*Western blot analysis*

After the cells were lysed, sonicated and homogenized in radioimmunoprecipitation (RIPA) buffer, protease inhibitors (1 mmol/L phenylmethanesulfonyl fluoride, PMSF) and phosphatase inhibitors (phosphatase inhibitor mixture) were added. The BCA Protein Assay Kit (Pierce, IL, USA) was used to determine the superalbumin concentration, and then proteins were electrophoresed with 12% SDS-PAGE and transferred to a membrane (Millipore Shanghai). Five percent nonfat milk was used to block membranes for 1 h and incubated with primary antibodies (1:1000 dilution), followed by a horseradish peroxidase-conjugated secondary antibody (1:10,000 dilution). The signal was detected by an enhanced chemiluminescence kit (Pierce, IL, USA). A digital image processing system (Universal Hood II76S/0608, Bio-Rad, Hercules, CA) was used to acquire images of blots, which were quantified using Quantity One (Bio-Rad).

*Statistical analysis*

The data were described as the mean ± SD and were analyzed using SPSS 22.0 software (SPSS Inc., Chicago, IL) by unpaired Student's t test or one-way ANOVA. Significant differences were determined by Duncan's multiple range tests. Diferences were considered significant at $P<0.05$.

## Results

*High uric acid activates cardiomyocyte mitophagy*

To determine the most suitable time and concentration for HUA treatment of cardiomyo-

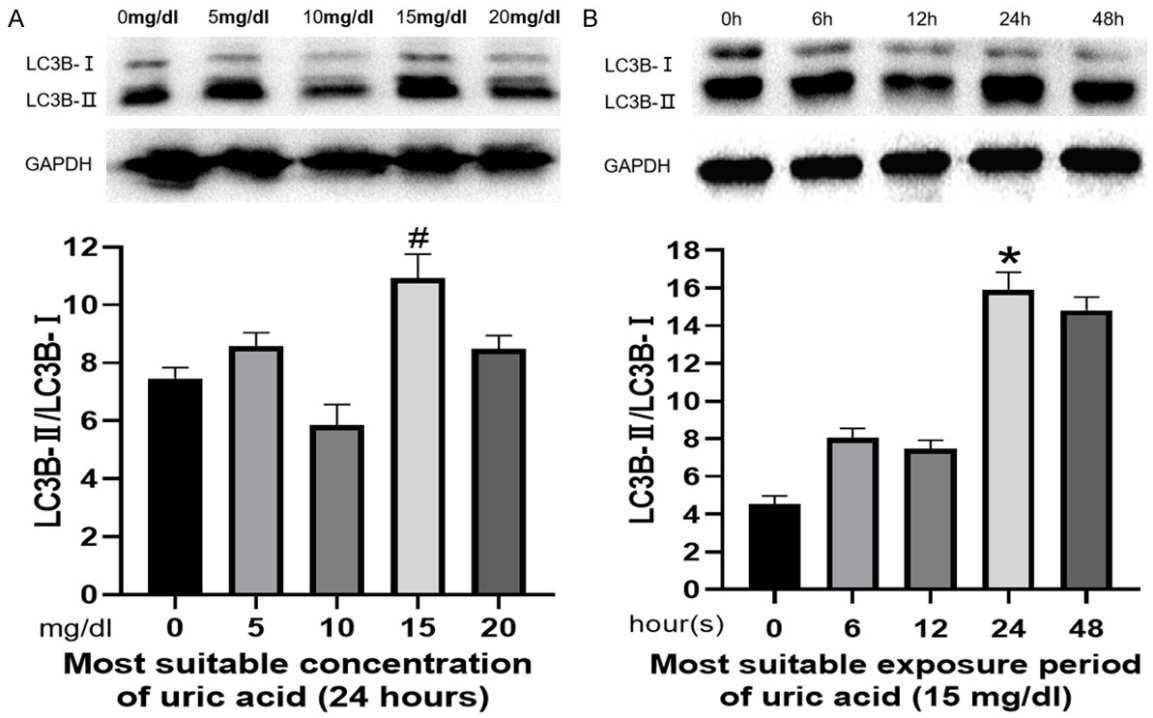


**Figure 1.** (A, B) Immunoblotting showing the expression of LC3B-II in cardiomyocytes to obtain the most appropriate concentration (A) and time (B). Quantification relative to GAPDH levels. *P<0.05 vs. 15 mg/dl, #P<0.05 vs. 0 h.

cytes, we applied gradients for time and concentration, and then the levels of mitophagy-related proteins LC3B-II were measured. HUA significantly increased the levels of the mitophagy-related protein LC3B-II after pretreatment with HUA (15 mg/dl) for 24 h. Therefore, we selected the time and concentration of 24 h and 15 mg/dl, respectively (**Figure 1A**, **1B**). To study the effect of HUA on mitophagy in cardiomyocytes, we measured mitochondrial morphological changes and the levels of mitophagy-related proteins under HUA treatment. Fluorescence imaging of the colocalization of lysosomes and mitochondria showed that the interaction between lysosomes and mitochondria was significantly increased under HUA treatment (**Figure 2A**). Similarly, the levels of the mitophagy-related proteins Parkin, CaMKIIδ and P62 were increased after treatment with HUA, indicating that treatment with HUA activates mitophagy in cardiomyocytes (**Figure 2B-D**).

*High uric acid causes oxidative stress and mitochondrial damage in cardiomyocytes*

To evaluate the effect of HUA on cardiomyocyte injury, ROS and ATP levels and MMP were detected. After treatment with HUA, flow cytometry indicated that the ROS level of cardiomyocytes was increased. In contrast, ROS production was reduced after treatment with the antioxidant NAC (**Figure 3A**). Similarly, pretreatment of cardiomyocytes with NAC prevented the HUA-mediated induction of LC3B-II and P62, indicating that ROS participated in HUA activation of mitophagy (**Figure 3B**, **3C**). Flow cytometry indicated that HUA treatment decreased MMP (**Figure 4A**, **4B**). Meanwhile, HUA treatment of cardiomyocytes caused ATP levels to decrease, indicating that HUA applied to cardiomyocytes caused oxidative stress, resulting in mitochondrial damage, but autophagy inhibition by cotreatment with 3-MA caused ATP levels to rise. The results suggest that HUA may be caused by excessive activation of mitophagy and mitochondria damage (**Figure 4C**).

*High uric acid activates mitophagy via the ROS/CaMKIIδ/Parkin pathway*

Previous research showed that Parkin was closely related to mitophagy [29]. To further clarify the pathways by which HUA activation leads to mitophagy, we pretreated cardiomyocytes with the autophagy inhibitor 3-MA before

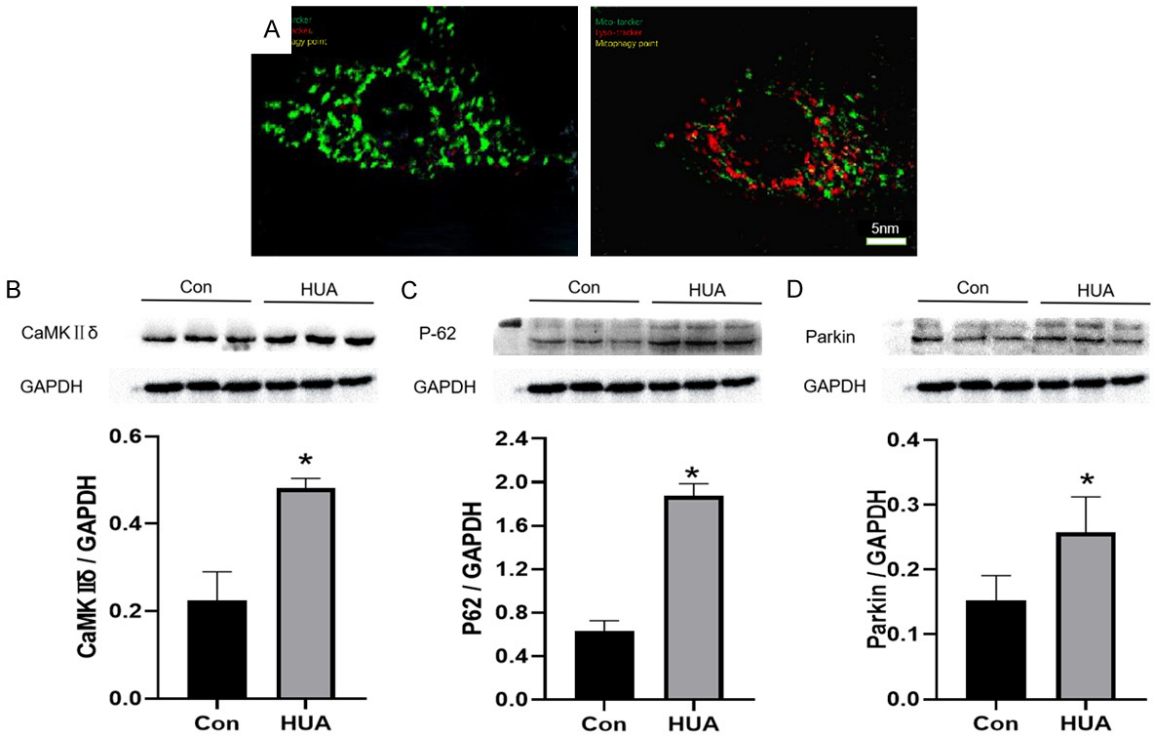


**Figure 2.** (A) H9c2 cardiomyocytes were treated with UA (uric acid) (15 mg/dl) for 24 h and then co-incubated with MitoTracker Green and LysoTracker Red to show lysosomal-mitochondrial interactions. Mitochondria are shown in green, lysosomes are shown in red, and mitophagy sites are in yellow. (B-D) Immunoblotting showing the expression of CaMKIIδ (B), P62 (C) and Parkin (D) in cardiomyocytes. Quantification relative to GAPDH levels. Data are presented as the mean ± SD (n=3). *P<0.05 vs. Control.

HUA treatment and then examined Parkin, LC3B-II and P62 protein levels. Pretreatment of cardiomyocytes with 3-MA prevented the HUA-mediated expression of Parkin and LC3B-II, whereas P62 levels continued to rise (**Figure 5A-C**). In addition, to assess whether calmodulin CaMKIIδ levels are involved in HUA activation of mitophagy, we pretreated cells with the calmodulin CaMKIIδ inhibitor KN-93 and revealed that CaMKIIδ levels were reduced and there was a decline in Parkin expression (**Figure 5D**, **5E**), indicating that calmodulin CaMKIIδ participated in HUA activation of mitophagy.

*Mitophagy is also activated in the murine acute model of hyperuricemia*

To determine whether HUA can also activate mitophagy *in vivo*, we established a murine model of acute hyperuricemia by applying HUA treatment for 24 h. Uric acid increased the expression of Parkin, LC3B-II and P62, and autophagy inhibition by pretreatment with 3-MA reduced Parkin and LC3B-II protein levels, whereas P62 levels continued to rise in cardiac tissue. Therefore, hyperuricemia also activated mitophagy *in vivo* (**Figure 6A-C**).

## Discussion

In our study, we explored the mechanism of HUA-induced mitophagy in cardiomyocytes. HUA may induce oxidative stress in cardiomyocytes, and oxidative stress plays an important role in the occurrence and development of mitophagy. The finding that HUA-increased parkin levels provides insight into one mechanism whereby HUA exposure can promote mitophagy signaling. Furthermore, CaMKIIδ-mediated $Ca^{2+}$ signaling-activated HUA is linked to mitophagy and mitochondrial dysfunction in cardiomyocytes (**Figure 7**).

As the pathophysiological basis of gout, many studies have shown that HUA is closely related to CVD [1-6], but the specific mechanism connecting uric acid and CVD is still unclear. Previous studies have unveiled that mitophagy plays a role in the progression of CVD, including heart failure [30], myocardial I/R injury [31],

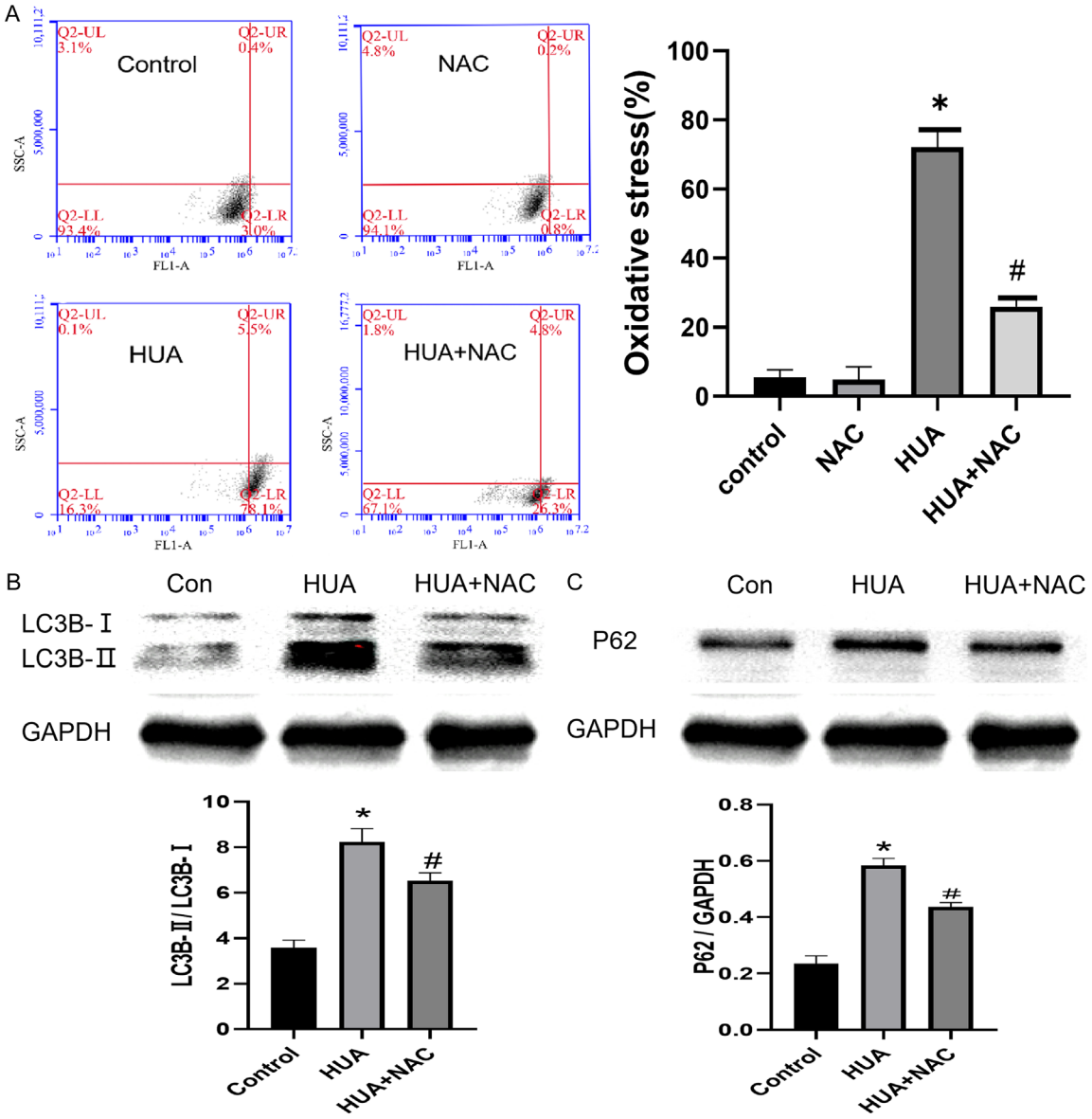


**Figure 3.** (A) Effect of HUA on ROS generation in H9c2 cardiomyocytes. Cells were co-incubated with HUA, stained with DCFH-DA and analyzed by flow cytometry. (B, C) Immunoblotting showing the expression of LC3B-II (B) and P62 (C) in cardiomyocytes. Quantification relative to GAPDH levels. Data are presented as the mean ± SD (n=3). *P<0.05 vs. control, #P<0.05 vs. HUA and N-acetyl-L-cysteine (NAC).

cardiomyocyte hypertrophy [32] and cardiomyopathy [33]. Our previous studies have shown that HUA increases oxidative stress in cardiomyocytes [8, 9]. In addition, oxidative stress is a major cause and mediator of mitophagy in many cell types [34, 35], including cardiomyocytes [10]. In this study, we proved that HUA could induce mitophagy directly in cardiomyocytes via oxidative stress. Importantly, the antioxidant NAC protected against HUA-induced mitophagy in cardiomyocytes, suggesting a major role of oxidative stress in HUA-induced mitophagy. Our results provide new evidence for HUA as an independent risk factor for CVD and a new explanation for how HUA affects the mitochondrial function of cardiomyocytes and is related to CVD. To the best of our knowledge, this is the first study to explain the role of HUA in cardiomyocyte damage via the regulation of mitophagy.

It's reported that CaMKII is also an important signaling molecule that regulates mitophagy [36]. Many studies have demonstrated that oxidative stress in response to cellular stress can cause a large number of calcium ions in cardio-

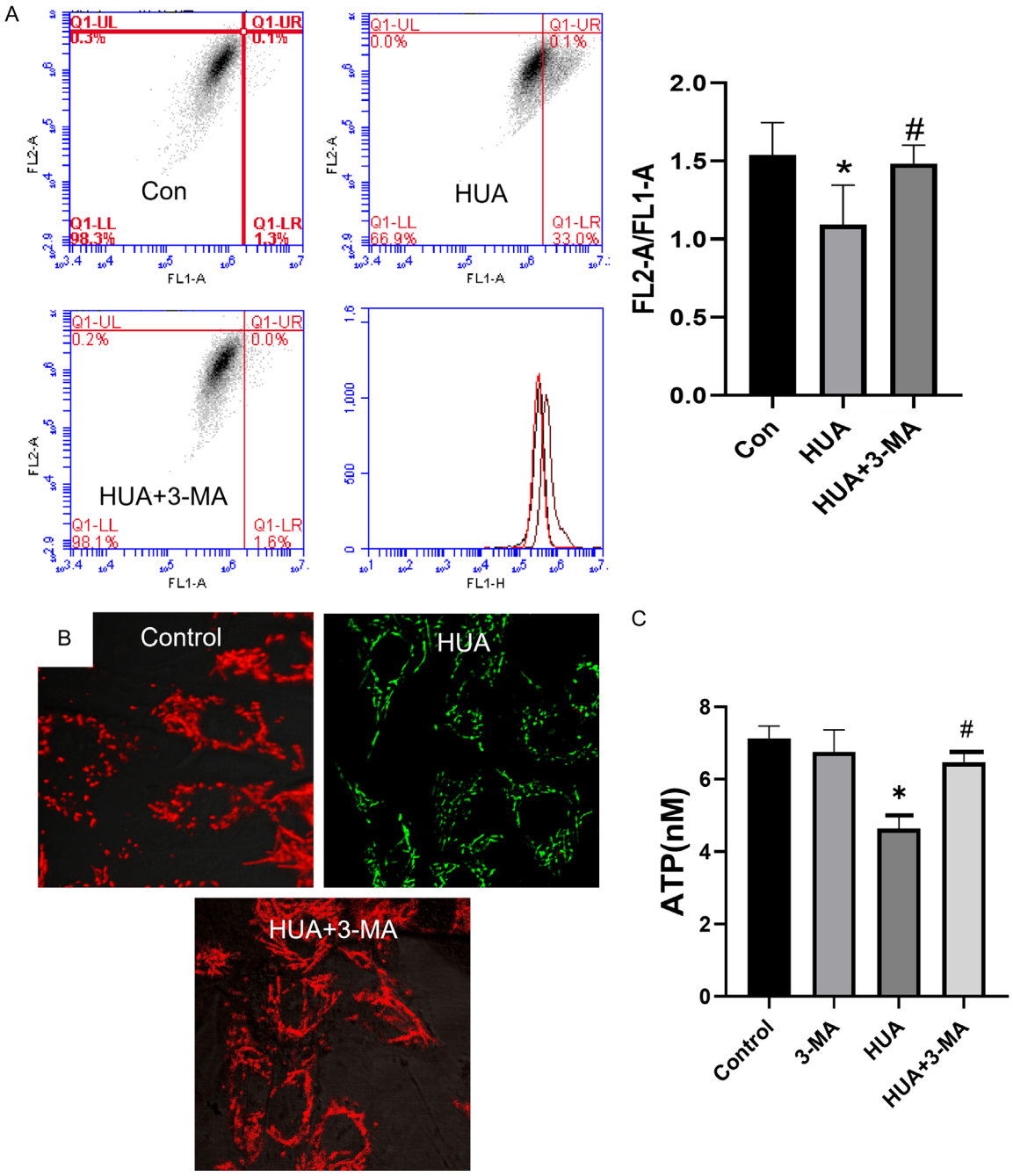


**Figure 4.** A. Cells were stained with PI and Annexin V-FITC and then subjected to flow cytometric analysis. B. Fluorescence images showing lysosomal-mitochondrial interactions. Mitochondria are shown in green, and lysosomes are shown in red. C. Intracellular ATP levels were determined by using an ATP assay kit with a luminometer. Data are presented as the mean ± SD (n=4). *P<0.01 vs. control, #P<0.01 vs. HUA, 3-methyladenine (3-MA).

myocytes to enter the mitochondria from the cytoplasm [21, 22], and oxidative stress directly activates CaMKII [37, 38]. Li et al. [39] reported that mitophagy can be directly activated by CaMKII in endothelial cells. Similarly, we found that the treatment of H9c2 cardiomyocytes with HUA increased CaMKIIδ and Parkin levels, suggesting that CaMKIIδ may be involved in mitophagy. In addition, to further verify the specific mechanism of CaMKII in mitophagy, we treated the cells with KN-93, an inhibitor of CaMKII. It's identified that the levels of CaMKIIδ

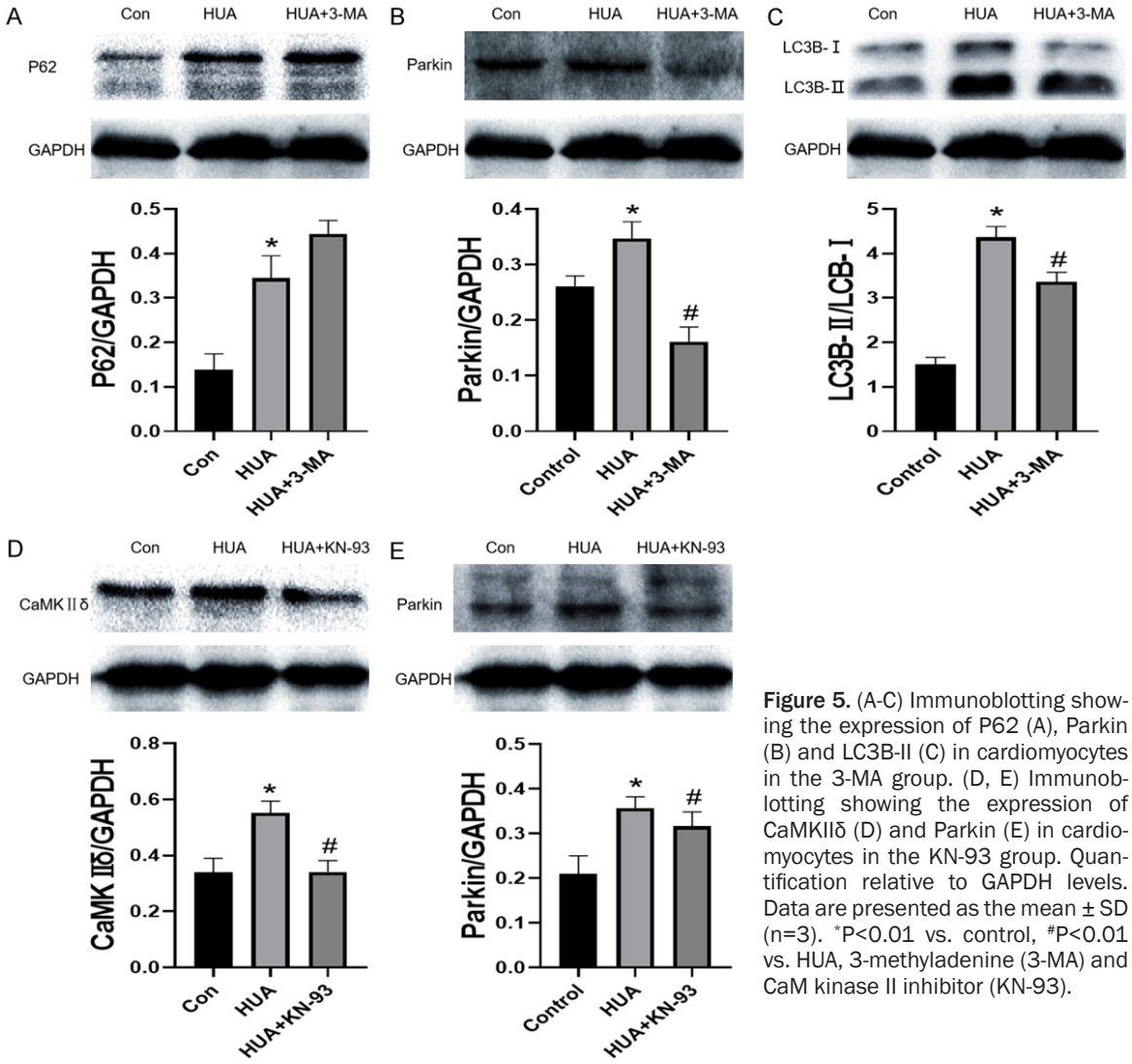


**Figure 5.** (A-C) Immunoblotting showing the expression of P62 (A), Parkin (B) and LC3B-II (C) in cardiomyocytes in the 3-MA group. (D, E) Immunoblotting showing the expression of CaMKIIδ (D) and Parkin (E) in cardiomyocytes in the KN-93 group. Quantification relative to GAPDH levels. Data are presented as the mean ± SD (n=3). *P<0.01 vs. control, #P<0.01 vs. HUA, 3-methyladenine (3-MA) and CaM kinase II inhibitor (KN-93).

and Parkin were decreased by KN-93, indicating that CaMKIIδ regulates the Parkin-mediated mitophagy caused by HUA.

Parkin is a crucial counter regulated mediator of the mitophagy pathway, which is the most studied and understood mitophagic pathway, at least in terms of mechanism [29, 34]. Moreover, Parkin-regulated mitophagy was activated when cardiomyocytes were subjected to various extracellular stresses. Catanzaro et al. [40] found that doxorubicin-induced cardiomyocyte death was mediated by Parkin-mitophagy. Wang et al. [41] showed that melatonin activated Parkin translocation and rescued the impaired mitophagy activity of diabetic cardiomyopathy through Mst1 inhibition. Similarly, in the present study, we found that treatment of H9c2 cardiomyocytes with HUA increased Parkin, LC3B-II and P62 levels. Importantly, the autophagy inhibitor 3-MA protected against HUA-induced mitophagy in cardiomyocytes, suggesting that Parkin plays a pivotal role in HUA-induced mitophagy under oxidative stress in cardiomyocytes. P62 is a key protein for mitophagy. Under normal mitophagy conditions, P62 is degraded with the progression of mitophagy. Intriguingly, in our study, exposure of cardiomyocytes to HUA also resulted in increased expression of P62. This signifies a possible blockade of mitophagic flux, leading to the accumulation of mitophagosomes. After NAC treatment, the P62 level was lower than that before the treatment in the HUA group, indicating that NAC prevents HUA activation and mitophagy and that ROS are involved in the process of mitophagy. After treatment with the autophagy inhibitor 3-MA, the P62 level did not

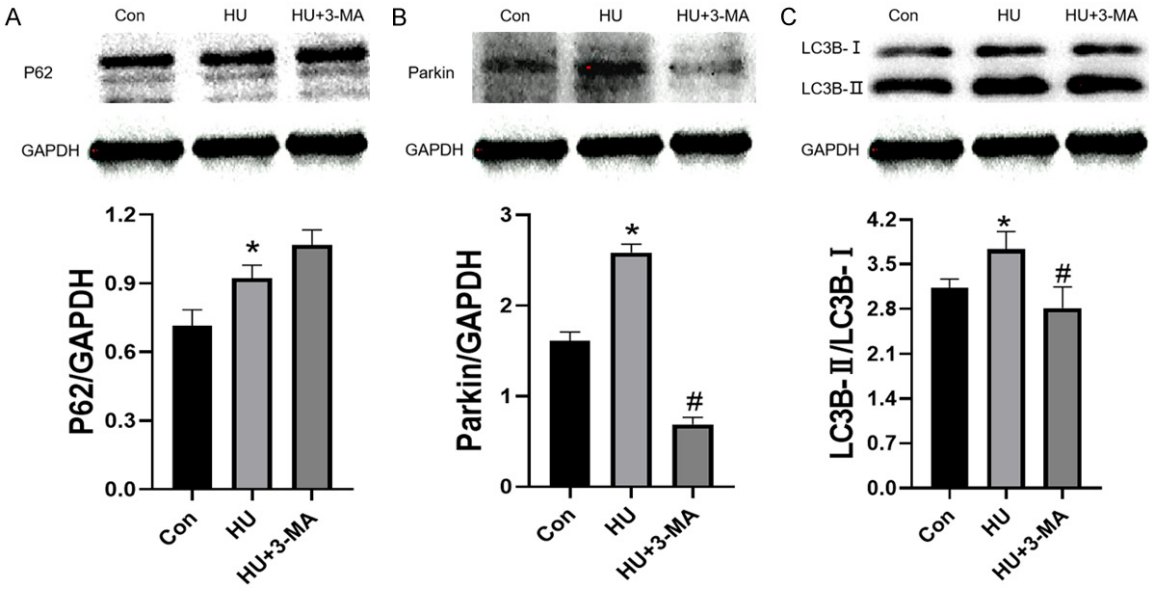


**Figure 6.** (A-C) Immunoblotting showing the expression of P62 (A), Parkin (B) and LC3B-II (C) in cardiac tissue. Quantification relative to GAPDH levels. Data are presented as the mean ± SD (n=3). *P<0.01 vs. control, #P<0.01 vs. HUA, 3-methyladenine (3-MA).

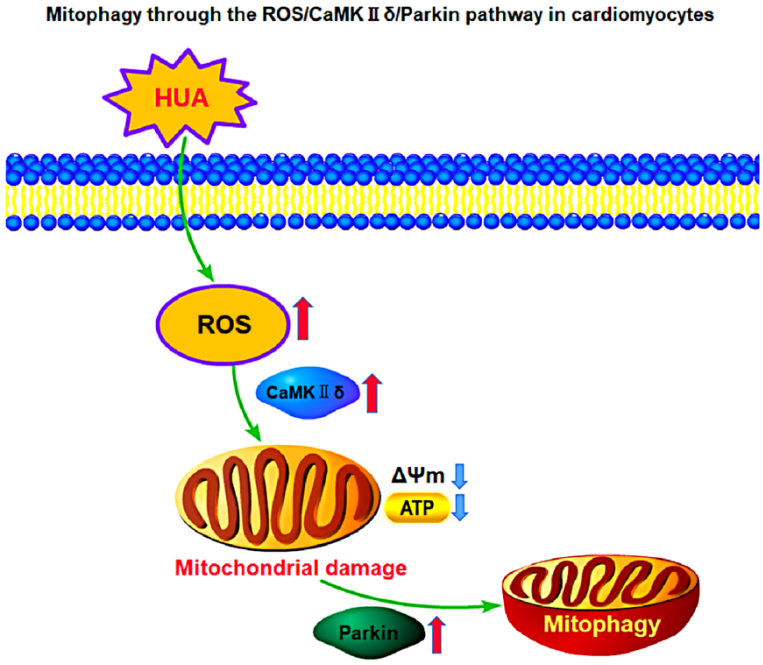


**Figure 7.** Schematic model of the mechanism underlying the regulatory role of ROS/CaMKIIδ/Parkinpathway in HUA-induced mitochondrial damage and mitophagy. This schematic model was made using Pathway Builder Tool 2.0.

decrease but rose further because 3-MA is mainly a specific inhibitor of PI3K and inhibits the conversion of LC3B-I to LC3B-II. This has also been verified in our study, and the addition of 3-MA cannot reverse the level of P62, but it inhibits the process of autophagy, increasing the accumulation of P62. This is in consistent with previous studies on mitophagy dysfunction after cell stress [42, 43]. HUA stimulation leads to the activation of mitophagy in cardiomyocytes. However, autophagy flow is inhibited, resulting in the failure of autophagy, an increase in P62, mitophagy dysfunction, and the accumulation of damaged mitochondria, which further causes cell damage and development of CVD. To verify whether mitochondrial injury occurred in mitophagy disorder induced by HUA, we measured MMP and ATP production. The results revealed that exposure to HUA significantly downregulated the MMP and impaired mitochondrial function, as evidenced by the ATP production rate, suggesting that HUA activates mitophagy in cardiomyocytes and causes mitochondrial damage and mitophagy disorders.

In the mouse model, the expression of CaMKIIδ and the mitophagy-related proteins Parkin, LC3B-II and P62 was increased in the experi-

mental group treated with HUA, while the expression of these proteins was decreased in the 3-MA treatment group compared with the experimental group. HUA can also activate mitophagy *in vivo*, as verified in animal models. No previous studies have shown that HUA is related to mitophagy in the myocardium, which may be a new therapeutic target for drugs.

In summary, HUA can promote cardiomyocyte mitophagy activation through the ROS/CaMKIIδ/Parkin pathway axis *in vivo* and *in vitro*. Exposure of cardiomyocytes to HUA leads to a significant increase in ROS levels and an increase in mitophagy initiation, but hinders mitophagy maturation, suggesting impaired mitophagy clearance and accumulation. The accumulation of damaged mitochondria containing mitotic bodies eventually causes mitochondrial damage in cardiomyocytes, decreases membrane potential, and reduces ATP production. Strategies to decrease uric acid levels may have prophylactic therapeutic potential for preventing HUA-induced cardiovascular dysfunction. The mechanism between hyperuricemia and CVD is further explained.

## Limitations

After treatment with HUA, we did not observe mitophagy by electron microscopy. Generally, electron microscopy is more intuitive. Second, we did not measure changes in the levels of Pink, another key protein in mitophagy [42]. When detecting autophagy flow, we only used the 3-MA and LC3B-II rather than chloroquine or bafilomycin A1 (autophagy flow inhibitors), the gold standard for testing the flow of autophagy. To date, we have only conducted experiments in animal models with acute high uric acid. In the future, our research will verify our experimental results in chronic models and even human models.

## Acknowledgements

Thanks to the central laboratory of Shantou University Medical College for providing the experimental site. The research was funded by Guangdong Basic and Applied Basic Research Foundation (2018A030307056) of China and Shantou Science and Technology Plan Project Foundation (180716084010717: [2018]155; [2019]106-1) of China.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Zhi Li, Department of Cardiology, The Second Affiliated Hospital of Shantou University Medical College, No. 69 Dongxia North Road, Jinping District, Shantou 515000, Guangdong Province, China. Tel: +86-13288767018; E-mail: lizhi519@126.com; Yang Xie, Emergency Department, The Second Affiliated Hospital of Shantou University Medical College, No. 69 Dongxia North Road, Jinping District, Shantou 515000, Guangdong Province, China. Tel: +86-13923660448; E-mail: xieyang2626@sina.com

## References

[1] Lazzeroni D, Bini M, Camaiora U, Castiglioni P, Moderato L, Bosi D, Geroldi S, Ugolotti PT, Brambilla L, Brambilla V and Coruzzi P. Serum uric acid level predicts adverse outcomes after myocardial revascularization or cardiac valve surgery. Eur J Prev Cardiol 2018; 25: 119-126.

[2] Xu Q, Zhang M, Abeysekera IR and Wang X. High serum uric acid levels may increase mortality and major adverse cardiovascular events in patients with acute myocardial infarction. Saudi Med J 2017; 38: 577-585.

[3] Peng D, Wang SP, Zhao DH, Fan QC, Shu J and Liu JH. Relationship between hyperuricemia and prognosis in patients with heart failure of coronary heart disease after revascularization. Zhonghua Yi Xue Za Zhi 2018; 98: 1337-1341.

[4] Lim SS, Yang YL, Chen SC, Wu CH, Huang SS, Chan WL, Lin SJ, Chen JW, Chou CY, Pan JP, Charng MJ, Chen YH, Wu TC, Lu TM, Hsu PF, Huang PH, Cheng HM, Huang CC, Sung SH, Lin YJ and Leu HB. Association of variability in uric acid and future clinical outcomes of patient with coronary artery disease undergoing percutaneous coronary intervention. Atherosclerosis 2020; 297: 40-46.

[5] Huang H, Huang B, Li Y, Huang Y, Li J, Yao H, Jing X, Chen J and Wang J. Uric acid and risk of heart failure: a systematic review and meta-analysis. Eur J Heart Fail 2014; 16: 15-24.

[6] Braga F, Pasqualetti S, Ferraro S and Panteghini M. Hyperuricemia as risk factor for coronary heart disease incidence and mortality in the general population: a systematic review and meta-analysis. Clin Chem Lab Med 2016; 54: 7-15.

[7] Borghi C, Tykarski A, Widecka K, Filipiak KJ, Domienik-Karłowicz J, Kostka-Jeziorny K, Varga A, Jaguszewski M, Narkiewicz K and Mancia G. Expert consensus for the diagnosis and treatment of patient with hyperuricemia and high

cardiovascular risk. Cardiol J 2018; 25: 545-563.
[8] Zhi L, Yuzhang Z, Tianliang H, Hisatome I, Yamamoto T and Jidong C. High uric acid induces insulin resistance in cardiomyocytes in vitro and in vivo. PLoS One 2016; 11: e0147737.
[9] Li Z, Shen Y, Chen Y, Zhang G, Cheng J and Wang W. High uric acid inhibits cardiomyocyte viability through the ERK/P38 pathway via oxidative stress. Cell Physiol Biochem 2018; 45: 1156-1164.
[10] Bartlett JJ, Trivedi PC and Pulinilkunnil T. Autophagic dysregulation in doxorubicin cardiomyopathy. J Mol Cell Cardiol 2017; 104: 1-8.
[11] Chistiakov DA, Shkurat TP, Melnichenko AA, Grechko AV and Orekhov AN. The role of mitochondrial dysfunction in cardiovascular disease: a brief review. Ann Med 2018; 50: 121-127.
[12] Truban D, Hou X, Caulfield TR, Fiesel FC and Springer W. PINK1, parkin, and mitochondrial quality control: what can we learn about Parkinson's disease pathobiology. J Parkinsons Dis 2017; 7: 13-29.
[13] Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, Kimura Y, Tsuchiya H, Yoshihara H, Hirokawa T, Endo T, Fon EA, Trempe JF, Saeki Y, Tanaka K and Matsuda N. Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature 2014; 510: 162-166.
[14] Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, Banerjee S and Youle RJ. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. J Cell Biol 2014; 205: 143-153.
[15] Zhang W, Chen C, Wang J, Liu L, He Y and Chen Q. Mitophagy in cardiomyocytes and in platelets: a major mechanism of cardioprotection against ischemia/reperfusion injury. Physiology (Bethesda) 2018; 33: 86-98.
[16] Qiao H, Ren H, Du H, Zhang M, Xiong X and Lv R. Liraglutide repairs the infarcted heart: the role of the SIRT1/Parkin/mitophagy pathway. Mol Med Rep 2018; 17: 3722-3734.
[17] Wang B, Nie J, Wu L, Hu Y, Wen Z, Dong L, Zou MH, Chen C and Wang DW. AMPKα2 protects against the development of heart failure by enhancing mitophagy via PINK1 phosphorylation. Circ Res 2018; 122: 712-729.
[18] Paradies G, Paradies V, Ruggiero FM and Petrosillo G. Mitochondrial bioenergetics and cardiolipin alterations in myocardial ischemia-reperfusion injury: implications for pharmacological cardioprotection. Am J Physiol Heart Circ Physiol 2018; 315: H1341-H1352.
[19] Kumar S, Wang G, Zheng N, Cheng W, Ouyang K, Lin H, Liao Y and Liu J. HIMF (hypoxia-induced mitogenic factor)-IL (interleukin)-6 signaling mediates cardiomyocyte-fibroblast crosstalk to promote cardiac hypertrophy and fibrosis. Hypertension 2019; 73: 1058-1070.
[20] Zhang P. CaMKII: the molecular villain that aggravates cardiovascular disease. Exp Ther Med 2017; 13: 815-820.
[21] Neef S, Steffens A, Pellicena P, Mustroph J, Lebek S, Ort KR, Schulman H and Maier LS. Improvement of cardiomyocyte function by a novel pyrimidine-based CaMKII-inhibitor. J Mol Cell Cardiol 2018; 115: 73-81.
[22] Yue R, Hu H, Yiu KH, Luo T, Zhou Z, Xu L, Zhang S, Li K and Yu Z. Lycopene protects against hypoxia/reoxygenation-induced apoptosis by preventing mitochondrial dysfunction in primary neonatal mouse cardiomyocytes. PLoS One 2012; 7: e50778.
[23] Hu J, Zhang Y, Jiang X, Zhang H, Gao Z, Li Y, Fu R, Li L, Li J, Cui H and Gao N. ROS-mediated activation and mitochondrial translocation of CaMKII contributes to Drp1-dependent mitochondrial fission and apoptosis in triple-negative breast cancer cells by isorhamnetin and chloroquine. J Exp Clin Cancer Res 2019; 38: 225.
[24] Zhao Q, Wang W and Cui J. Melatonin enhances TNF-α-mediated cervical cancer HeLa cells death via suppressing CaMKII/Parkin/mitophagy axis. Cancer Cell Int 2019; 19: 58.
[25] Li P, Bai Y, Zhao X, Tian T, Tang L, Ru J, An Y and Wang J. NR4A1 contributes to high-fat associated endothelial dysfunction by promoting CaMKII-Parkin-mitophagy pathways. Cell Stress Chaperones 2018; 23: 749-761.
[26] Barbu A, Welsh N and Saldeen J. Cytokine-induced apoptosis and necrosis are preceded by disruption of the mitochondrial membrane potential (Deltapsi(m)) in pancreatic RINm5F cells: prevention by Bcl-2. Mol Cell Endocrinol 2002; 190: 75-82.
[27] Wang C, Liu N, Luan R, Li Y, Wang D, Zou W, Xing Y, Tao L, Cao F and Wang H. Apelin protects sarcoplasmic reticulum function and cardiac performance in ischaemia-reperfusion by attenuating oxidation of sarcoplasmic reticulum Ca2+-ATPase and ryanodine receptor. Cardiovasc Res 2013; 100: 114-124.
[28] Li JM, Zhang X, Wang X, Xie YC and Kong LD. Protective effects of cortex fraxini coumarines against oxonate-induced hyperuricemia and renal dysfunction in mice. Eur J Pharmacol 2011; 666: 196-204.
[29] Niu K, Fang H, Chen Z, Zhu Y, Tan Q, Wei D, Li Y, Balajee AS and Zhao Y. USP33 deubiquitinates PRKN/parkin and antagonizes its role in mitophagy. Autophagy 2020; 16: 724-734.
[30] Wang B, Nie J, Wu L, Hu Y, Wen Z, Dong L, Zou MH, Chen C and Wang DW. AMPKα2 protects against the development of heart failure by en-

hancing mitophagy via PINK1 phosphorylation. Circ Res 2018; 122: 712-729.
[31] Yao L, Chen H, Wu Q and Xie K. Hydrogen-rich saline alleviates inflammation and apoptosis in myocardial I/R injury via PINK-mediated autophagy. Int J Mol Med 2019; 44: 1048-1062.
[32] Pennanen C, Parra V, López-Crisosto C, Morales PE, Del Campo A, Gutierrez T, Rivera-Mejías P, Kuzmicic J, Chiong M, Zorzano A, Rothermel BA and Lavandero S. Mitochondrial fission is required for cardiomyocyte hypertrophy mediated by a Ca2+-calcineurin signaling pathway. J Cell Sci 2014; 127: 2659-2671.
[33] Tong M, Saito T, Zhai P, Oka SI, Mizushima W, Nakamura M, Ikeda S, Shirakabe A and Sadoshima J. Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. Circ Res 2019; 124: 1360-1371.
[34] Lin Q, Li S, Jiang N, Shao X, Zhang M, Jin H, Zhang Z, Shen J, Zhou Y, Zhou W, Gu L, Lu R and Ni Z. PINK1-parkin pathway of mitophagy protects against contrast-induced acute kidney injury via decreasing mitochondrial ROS and NLRP3 inflammasome activation. Redox Biol 2019; 26: 101254.
[35] Yau WW, Singh BK, Lesmana R, Zhou J, Sinha RA, Wong KA, Wu Y, Bay BH, Sugii S, Sun L and Yen PM. Thyroid hormone (T(3)) stimulates brown adipose tissue activation via mitochondrial biogenesis and MTOR-mediated mitophagy. Autophagy 2019; 15: 131-150.
[36] Zhao Q, Wang W and Cui J. Melatonin enhances TNF-α-mediated cervical cancer HeLa cells death via suppressing CaMKII/Parkin/mitophagy axis. Cancer Cell Int 2019; 19: 58.
[37] Zhu LJ, Klutho PJ, Scott JA, Xie L, Luczak ED, Dibbern ME, Prasad AM, Jaffer OA, Venema AN, Nguyen EK, Guan X, Anderson ME and Grumbach IM. Oxidative activation of the Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) regulates vascular smooth muscle migration and apoptosis. Vascul Pharmacol 2014; 60: 75-83.
[38] Zhang Y, Zhang L, Zhang Y, Fan X, Yang W, Yu B, Kou J and Li F. YiQiFuMai powder injection attenuates coronary artery ligation-induced heart failure through improving mitochondrial function via regulating ros generation and CaMKII signaling pathways. Front Pharmacol 2019; 10: 381.
[39] Li P, Bai Y, Zhao X, Tian T, Tang L, Ru J, An Y and Wang J. NR4A1 contributes to high-fat associated endothelial dysfunction by promoting CaMKII-Parkin-mitophagy pathways. Cell Stress Chaperones 2018; 23: 749-761.
[40] Catanzaro MP, Weiner A, Kaminaris A, Li C, Cai F, Zhao F, Kobayashi S, Kobayashi T, Huang Y, Sesaki H and Liang Q. Doxorubicin-induced cardiomyocyte death is mediated by unchecked mitochondrial fission and mitophagy. FASEB J 2019; 33: 11096-11108.
[41] Wang S, Zhao Z, Feng X, Cheng Z, Xiong Z, Wang T, Lin J, Zhang M, Hu J, Fan Y, Reiter RJ, Wang H and Sun D. Melatonin activates Parkin translocation and rescues the impaired mitophagy activity of diabetic cardiomyopathy through Mst1 inhibition. J Cell Mol Med 2018; 22: 5132-5144.
[42] Zhang Y, Xi X, Mei Y, Zhao X, Zhou L, Ma M, Liu S, Zha X and Yang Y. High-glucose induces retinal pigment epithelium mitochondrial pathways of apoptosis and inhibits mitophagy by regulating ROS/PINK1/Parkin signal pathway. Biomed Pharmacother 2019; 111: 1315-1325.
[43] Thangaraj A, Periyasamy P, Liao K, Bendi VS, Callen S, Pendyala G and Buch S. HIV-1 TAT-mediated microglial activation: role of mitochondrial dysfunction and defective mitophagy. Autophagy 2018; 14: 1596-1619.