Cinnamaldehyde regulates mitochondrial quality against hydrogen peroxide induced apoptosis in mouse lung mesenchymal stem cells *via* the PINK1/Parkin signaling pathway

Shiwen Ke[1], Wei Zhu[2], Zhihui Lan[1], Yuanbing Zhang[1], Lisha Mo[1], Guoshuang Zhu[1] and Liangji Liu[1]

[1] Department of Respiration, Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Nanchang, Jiangxi, China

[2] The Second Clinical Medical School, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, China

ABSTRACT

Background. Idiopathic pulmonary fibrosis (IPF) is a fatal respiratory disease without effective treatments. Mitochondrial dysfunction weakens the ability of mesenchymal stem cells (MSCs) to repair the distal lung epithelium, which is a probable pathogenesis of IPF. In previous research, we found that cinnamaldehyde (CA) can maintain the mitochondrial morphology of MSCs.

Methods. This present study evaluated the effect and mechanism of CA on murine lung MSCs using the hydrogen peroxide model. Antioxidant effects and mitochondrial function were determined using flow cytometry. The mRNA levels of mitochondrial dynamics and the expressions of autophagy-related proteins were also detected.

Results. CA can increase the levels of SOD, MMP and ATP, decrease the rate of ROS and apoptosis, and restore the mitochondrial structure. CA can also improve the mRNA expression of MFN1, MFN2, FIS1, DRP1, OPA1, and PGC-1α, increase the expression of LC3 II and p62 and promote the PINK1/Parkin signaling pathway. Our results demonstrated that CA can control mitochondrial quality and avoid apoptosis, which may be associated with the regulation of the PINK1/Parkin signaling pathway.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive respiratory disease with a median survival time of 2.8 years (*Xaubet, Ancochea & Molina-Molina, 2017*). Nintedanib and pirfenidone are approved IPF treatments, but neither drug has prospectively shown a survival benefit (*Richeldi, Collard & Jones, 2017*). Mesenchymal stem cells (MSCs) have attracted attention as a possible treatment for IPF in recent years (*Averyanov et al., 2020*; *Tzouvelekis et al., 2018*). There is a connection between the mitochondrial dysfunction of MSCs and IPF (*Cárdenes et al., 2018*). Mitochondrial quality affects the differentiation,

Corresponding author
Liangji Liu, llj6505@163.com

repairing function and survival of MSCs (*Li et al., 2017*). Therefore, avoiding mitochondrial dysfunction, regulating the vitality of MSCs, and preserving their repairing effects on lung injuries may be a safer and better treatment method for IPF.

Cinnamaldehyde (CA) is a kind of monomer derived from *Cinnamomum cassia* with many biological properties, including antioxidant and anti-inflammatory properties (*Doyle & Stephens, 2019*; *Ismail et al., 2022*). The antioxidant effects of CA are widely recognized and it has even recently been used as a cancer-specific oxidative stress amplification nanomedicine (*Wang et al., 2022a*). One study suggested that CA can affect the viability, growth, and differentiation of human adipose-derived MSCs (*Absalan et al., 2016*). Additionally, several studies have shown the close relationship between CA and the mitochondria (*Han et al., 2020*; *Choi, 2021*). In previous research, we found that CA can maintain the mitochondrial morphology of MSCs. Therefore, we propose the following scientific hypotheses: CA can regulate the mitochondrial quality and enhance the vitality of MSCs. In this study, the effects of CA on murine lung mesenchymal stem cells (LMSCs) were investigated and its possible mechanisms were explored.

MATERIALS & METHODS

Cell extraction and identification

LMSCs were extracted from C57BL/6 mice and the cells were identified using flow cytometry. Healthy male C57BL/6 mice were provided by the Jiangxi University of Traditional Chinese Medicine (Jiangxi, China). Mice were reared in a barrier environment for one week and then euthanized by cervical dislocation after anesthesia with phenobarbital. Surviving animals continued to be reared for subsequent cell extraction. Mice were also euthanized if they developed depression, loss of appetite, or an inability to stand. More details are provided in Files S1.1–S1.2. This study was approved by the Ethics Committee for Animal Experimentation of Jiangxi University of Traditional Chinese Medicine (SYXK2017-0004).

Antioxidant effects of CA

In this study, hydrogen peroxide (H_2O_2) was used for modelling. The antioxidant effects of CA on cells were evaluated using measurements of cell viability, total SOD level, intracellular ROS level and apoptosis rate. N-Acetyl-L-cysteine (NAC), the classical antioxidant, was used as a positive control (Files S1.3–S1.6).

Study of cellular mitochondrial quality

The mitochondrial quality of murine LMSCs was assessed using the levels of mitochondrial membrane potential and ATP, and mitochondrial morphology was observed using a transmission electron microscope. The details are displayed in Files S1.7–S1.9.

Investigations of the expression levels of related mRNA and protein

The mRNA levels of mitochondrial dynamics and the expression levels of autophagy-related proteins were then measured and the preliminary exploration of possible mechanisms was carried out. The detailed methods of PCR and Western blotting used for these measurements are provided in Files 1.10–S1.11.

Statistical analysis

All experimental data were obtained from at least three independent experiments and presented as mean ± standard deviation, and between-groups data were evaluated using a one-way ANOVA test on GraphPad software version 8.0 (GraphPad, San Diego, CA, USA). $P < 0.05$ was defined as statistically different.

RESULTS

Molecular characterization of CA and cell identification

The molecular structure of CA is shown in Fig. 1A, and its molecular weight is 132.16. In addition, the microscopic morphology of murine LMSCs is shown in Fig. 1B (Files S2.1). Flow cytometry results showed that CD44, CD54, CD90, CD105, and Sca-1 were expressed, while CD11b and CD45 were not (Fig. 1C). These results are consistent with the phenotypic characteristics of MSCs.

Antioxidant effects of CA on H_2O_2-induced murine LMSCs

The survival rate of mouse LMSCs decreased gradually as the H_2O_2 dose increased (Fig. 2A), but the survival rates improved with a 50–400 µM CA intervention (Fig. 2B). The levels of SOD are shown in Fig. 2C; SOD levels in the CA and NAC groups were significantly higher than in the H_2O_2 group. ROS levels in the CA and NAC groups groups were significantly lower than in the H_2O_2 group (Fig. 2D). The apoptosis rates of the CA and NAC groups were also significantly lower than the H_2O_2 group (Fig. 2E). Among the above three indicators, the antioxidant capacity of 200 µM CA on H_2O_2-induced murine LMSCs was superior to that of NAC. More data are documented in Files S2.2–S2.5.

Protective effects of CA on mitochondrial function

The MMP levels of the NAC and CA groups were significantly higher than the H_2O_2 group, and the level of the 200 µM CA group was higher than that of the NAC group (Fig. 3A). Additionally, the ATP levels of the 200 µM CA and NAC groups were obviously higher than in the H_2O_2 groups (Fig. 3B). The detailed data are presented in Files S2.6–S2.7.

Effects of CA on mitochondrial morphology

Photos of the cells taken by transmission electron microscopy are provided in Fig. 3C. The mitochondria in the control group were healthy, mostly oval in shape, with clear cristae and intact membranes. The mitochondrial structure was seriously damaged in the H_2O_2 groups: the shape was deformed and twisted; the cristae of the mitochondria were loose, broken or missing; the density of the matrix was decreased or uneven; and numerous vacuoles were observed. In the 50 µM CA and NAC groups, the morphology of the mitochondria was still abnormal, but the cristae could be distinguished, and the matrix was relatively uniform. The morphology in the 200 µM CA group was relatively close to the control group, but with autophagosomes found in the 200 µM CA group.

Effects of CA on the mRNA levels of mitochondrial dynamics

Compared with the control groups, the mRNA levels of FIS1, MFN1, MFN2, DRP1, OPA1, and PGC-1α were decreased in the H_2O_2 groups. All mRNA levels in the 200 µM CA
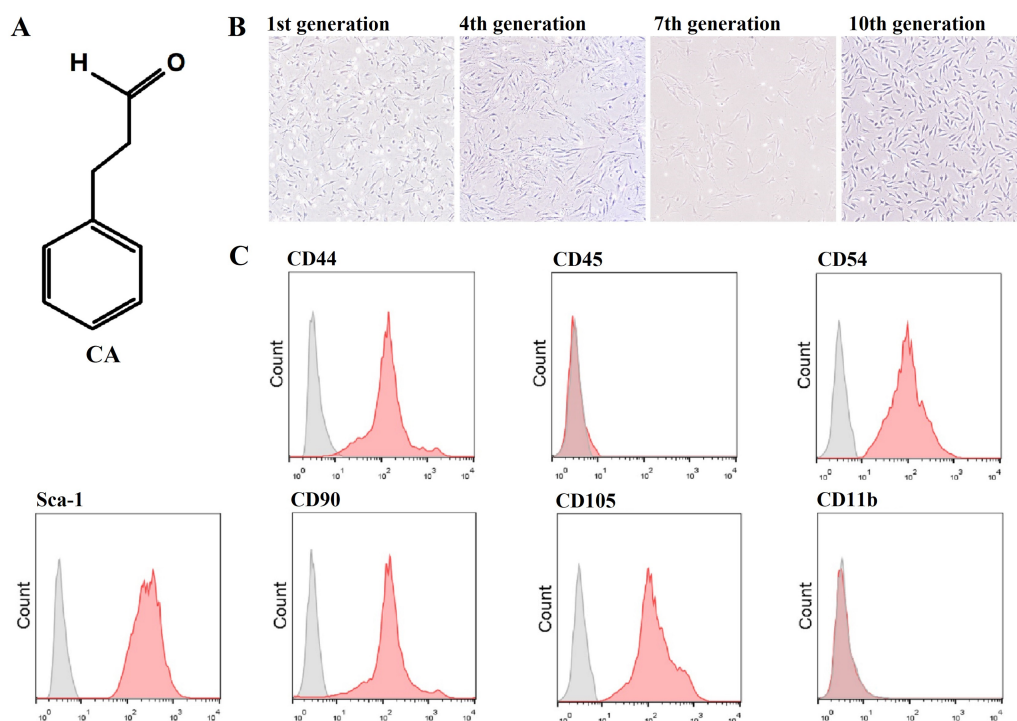
Figure 1 **Molecular characterization of cinnamaldehyde (CA) and identification of murine LMSCs.**
(A) Chemical structure of CA. (B) Microscopic morphology of murine LMSCs (magnification: 4×). (C)
Phenotypes of mouse LMSCs.

groups were obviously higher than those in the H_2O_2 groups, but there was no significant
difference in the mRNA levels of FIS1, MFN1, DRP1, and OPA1 between the NAC and
H_2O_2 groups. These results are represented in Fig. 4A. (Files S2.8).

Effects of CA on the expression levels of autophagy-related proteins

There was no statistical difference in the expression of Beclin-1 in all groups (Fig. 4B).
There was also no obvious difference in the levels of LC3-II in the H_2O_2 and NAC groups
compared with the control group (Fig. 4C), but the level of LC3-II in the 200 μM CA
group was markedly higher than in the control group. P62 levels (Fig. 4D) were lower in
the H_2O_2 group compared to the control group. There was no significant difference in P62
levels between the NAC and H_2O_2 groups, but the P62 level in the 200 μM CA group was
significantly higher. These data are presented in Files S2.9.

Effects of CA on the PINK1/Parkin pathway of H_2O_2-treated murine LMSCs

Compared with the control groups, the mRNA levels of PINK1 and Parkin were decreased
in the H_2O_2 groups. However, all the mRNA levels in the 200 μM CA groups were obviously
increased (Fig. 5A). The protein expression level of PINK1 in the H_2O_2 group was higher
than the expression level in the control group, and the expression level of PINK1 in the
200 μM CA group was higher than in the H_2O_2 group (Fig. 5B). Moreover, the protein
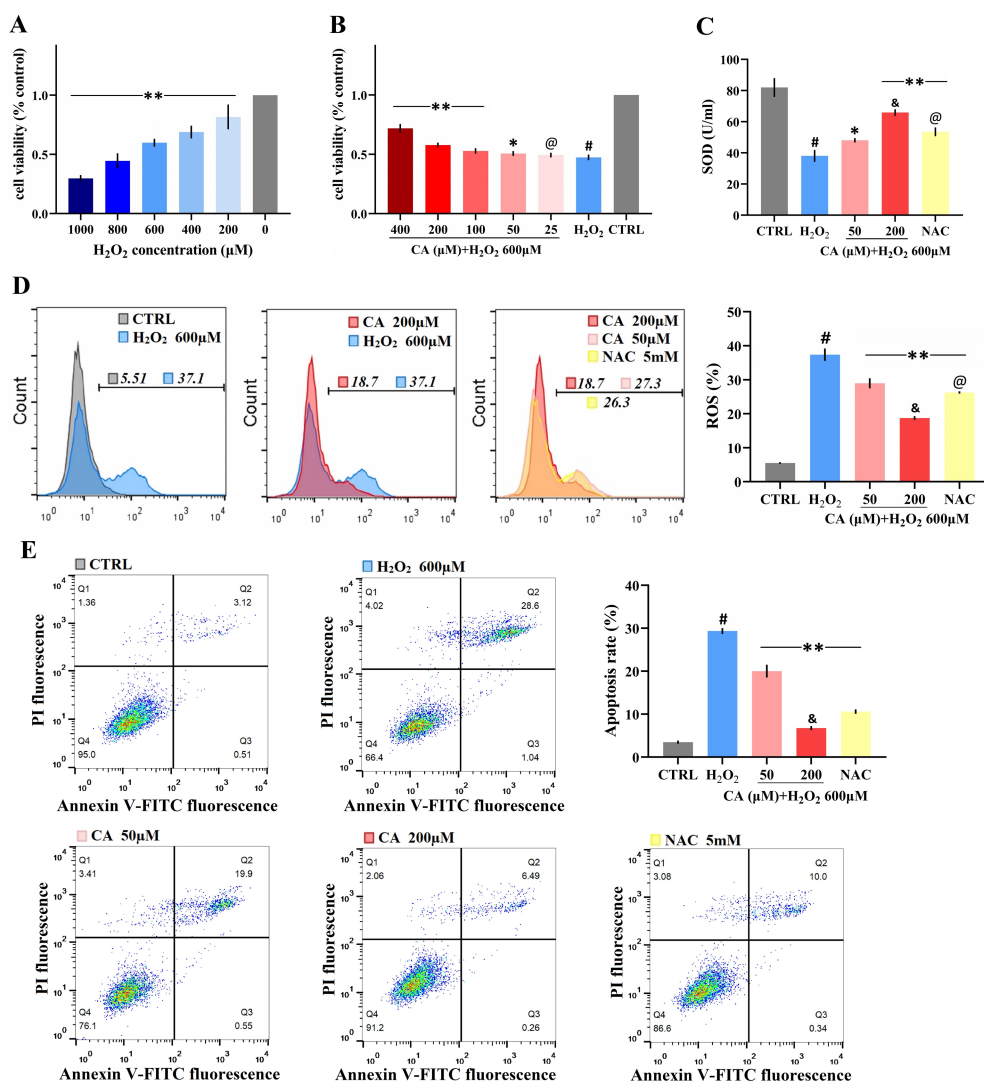
Figure 2 Antioxidant effects of CA on H$_2$O$_2$-induced murine LMSCs. (A) The effects of various doses of H$_2$O$_2$ on cell viability. Data were obtained from seven independent experiments; **$P < 0.01$ versus 0 μM H$_2$O$_2$ group. (B) The protective effects of CA on H$_2$O$_2$-induced cells. Data were obtained from nine independent experiments; @$P > 0.05$, *$P < 0.05$, and **$P < 0.01$ versus H$_2$O$_2$ group. (C) Total SOD levels of groups. (D) Intracellular ROS production of each group. (E) Apoptosis rates of groups. Data were obtained from three independent experiments; #$P < 0.01$ versus CTRL groups; *$P < 0.05$, **$P < 0.01$ versus H$_2$O$_2$ groups; &$P < 0.05$ versus NAC groups; @$P > 0.05$ versus 50 μM CA groups.

expression level of Parkin in the H$_2$O$_2$ group was lower than the level in the control group, and the expression level in the 200 μM CA group was higher than in the H$_2$O$_2$ group (Fig. 5C). More data are presented in Files S2.10.

Peer J
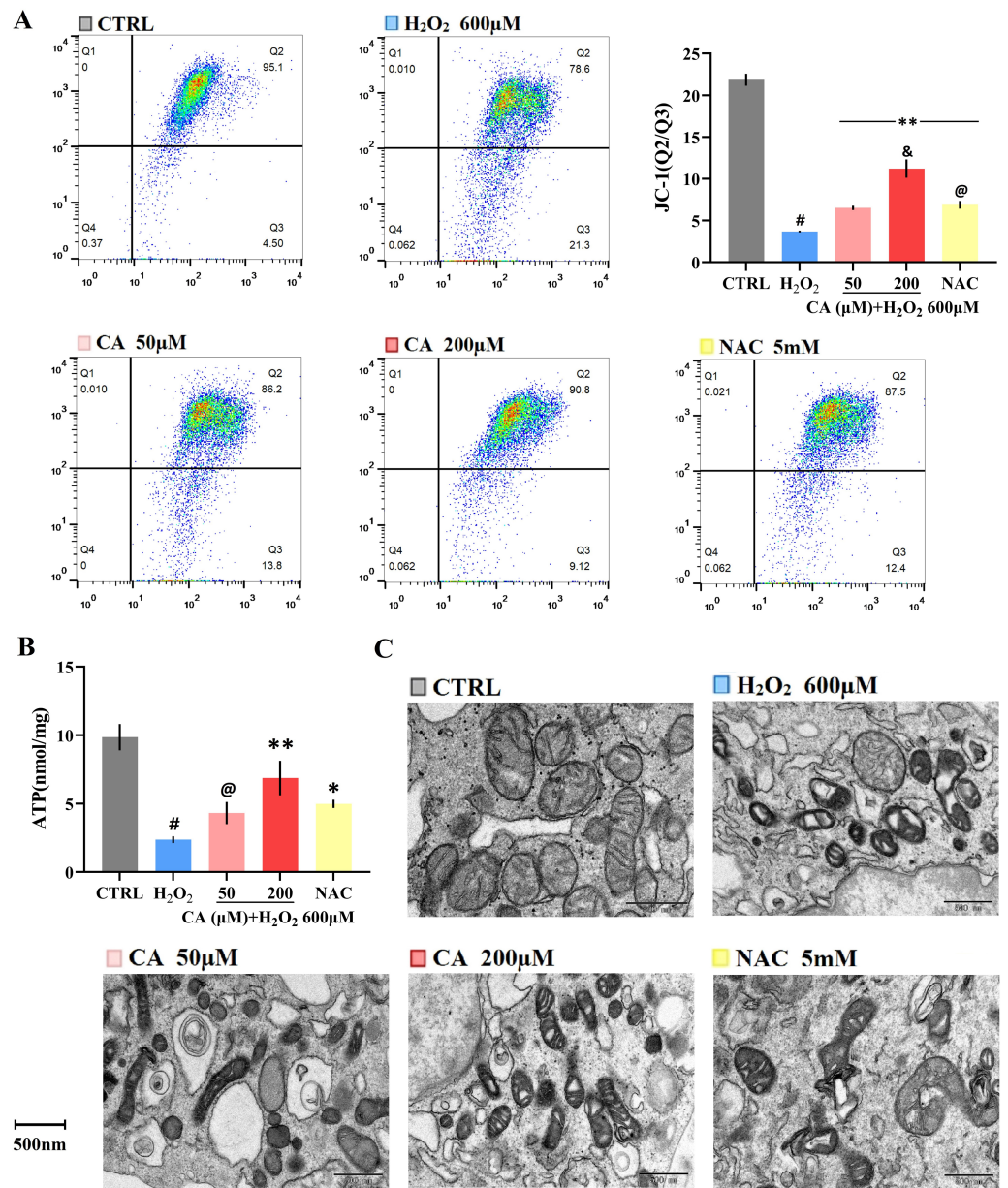
Figure 3 Effects of CA on mitochondrial function and morphology of H_2O_2-treated murine LMSCs.
(A) The MMP levels of cells; #$P < 0.01$ versus CTRL group. Data were obtained from three independent experiments; **$P < 0.01$ versus H_2O_2 group; &$P < 0.05$ versus NAC group; @$P > 0.05$ versus 50 μM CA group. (B) Cellular ATP production of each group. Data were obtained from three independent experiments; #$P < 0.01$ versus CTRL group; *$P < 0.05$, **$P < 0.01$ versus H_2O_2 group; @$P > 0.05$ versus NAC group. (C) The mitochondria were observed by transmission electron microscopy (magnification: 14,000×).
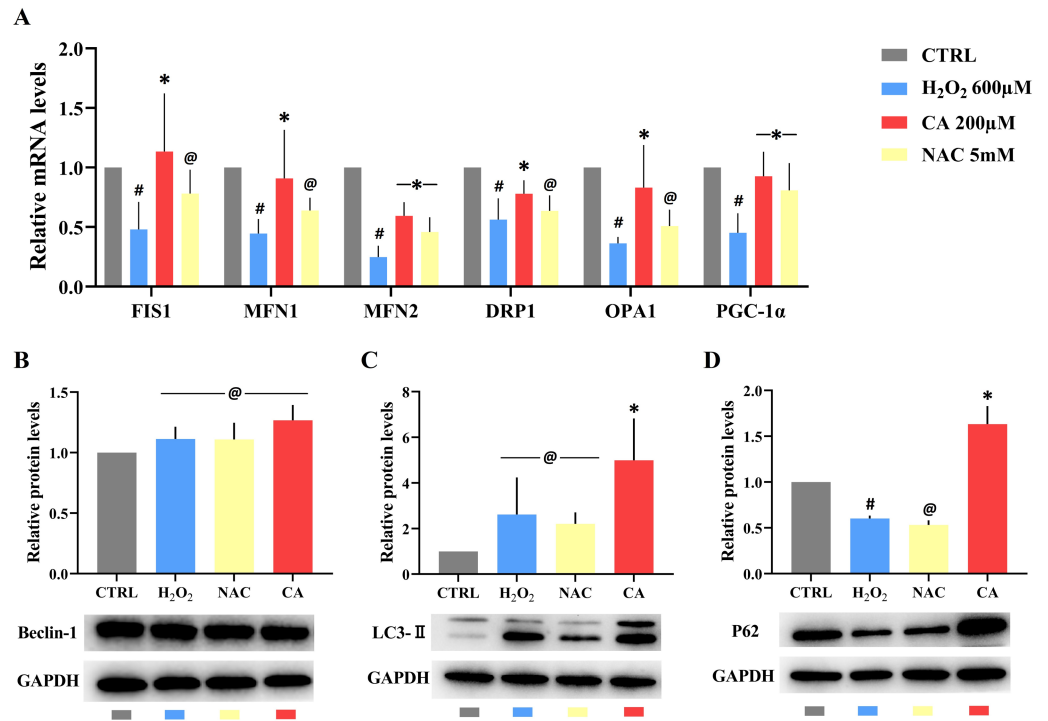
Figure 4 **Effects of CA on mitochondrial dynamics genes and autophagy-related proteins in H_2O_2-treated murine LMSCs.** (A) The mRNA levels of FIS1, MFN1, MFN2, DRP1, OPA1 and PGC-1 α in groups. Data were obtained from six independent experiments; $^{\#}P < 0.05$ versus CTRL groups; $^{\star}P < 0.05$, $^{@}P > 0.05$ versus H2O2 groups. (B) The protein expression levels of Beclin-1; $^{@}P > 0.05$ versus CTRL group. (C) The protein expression levels of LC3-II; $^{@}P > 0.05$ versus CTRL group, $^{\star}P < 0.05$ versus H_2O_2 group. (D) The protein expression levels of P62; $^{\#}P < 0.05$ versus CTRL group; $^{\star}P < 0.05$, $^{@}P > 0.05$ versus H_2O_2 group. Protein expression levels were obtained from three independent experiments.
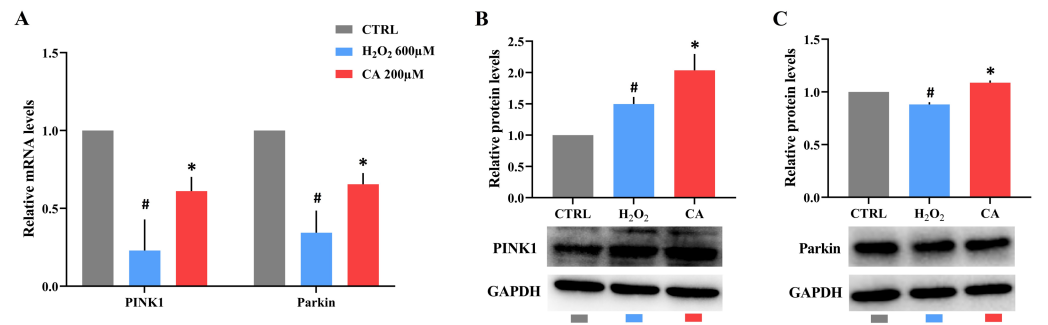
Figure 5 **Effects of CA on the PINK1/Parkin pathway of H_2O_2-treated murine LMSCs.** (A) The mRNA levels of PINK1 and Parkin in groups. (B) The protein expression levels of PINK1 in groups. (C) The protein expression levels of Parkin in groups. $^{\#}P < 0.05$ versus CTRL groups; $^{\star}P < 0.05$ versus H_2O_2 groups. Data were obtained from three independent experiments.

DISCUSSION

IPF is mainly manifested as progressive pulmonary fibrosis, in which healthy and soft lung tissue are replaced by diseased and stiff scars, ultimately endangering the life of the patient (*Martinez et al., 2017*). The connection between mitochondrial dysfunction and IPF has been a hot topic in recent years (*Cárdenes et al., 2018*). Mitochondrial dysfunction weakens the ability of MSCs to repair the distal lung epithelium, which is one of the key factors in the pathogenesis of IPF (*Fergie et al., 2019*). We first found that CA can control mitochondrial quality and avoid the apoptosis of LMSCs, which may be associated with the regulation of the PINK1/Parkin signaling pathway.

As the energy factories of cells, mitochondria produce most of the ATP required for cells. The potential difference between the two sides of the mitochondrial membrane is called the mitochondrial membrane potential (MMP), and is the basis for maintaining mitochondrial oxidation and ATP production. When the quality of mitochondria become worse, MMP and ATP levels decline (*Van der Bliek, Sedensky & Morgan, 2017*). We observed that CA can maintain MMP levels and increase ATP content in LMSCs. We also obtained more evidence from morphology that CA restored the mitochondrial structure of H_2O_2-induced LMSCs and the number of mitochondria was increased. Therefore, CA is beneficial to the function and morphology of mitochondria. Moreover, autophagosomes were found in the cells treated by CA. This means that CA is likely to maintain mitochondrial quality through the promotion of mitochondrial autophagy.

The occurrence of mitophagy depends on mitochondrial dynamics, including mitochondrial fission and fusion (*Onishi et al., 2021*). Mitochondria weed out impaired mitochondria that need to be cleared through continuous fusion and division, and then induce mitochondrial autophagy (*Wai & Langer, 2016*). The selective removal of damaged mitochondria by autophagosomes is the key to mitochondrial quality control (*Kubli & Gustafsson, 2012*). Mitochondrial dynamics are co-regulated by the MFN1, MFN2, FIS1, DRP1, and OPA1 proteins. We found that CA can improve the mRNA expressions of these proteins. MFN1 and MFN2 are located on the outer mitochondrial membrane and induce the fusion of the outer mitochondrial membrane (*Song et al., 2017*). DRP1 mainly mediates mitochondrial division and usually exists in the mitochondrial matrix. DRP1 proteins are recruited by FIS1 on the outer membrane to complete mitochondrial division together (*Ikeda et al., 2015*). OPA1 is located in the inner mitochondrial membrane and mediates the fusion of the inner membrane (*Rovira-Llopis et al., 2017*). However, the expression patterns of the genes related to mitochondrial fission and fusion were the same in this experiment, which differs from the opposite patterns commonly seen in studies related to mitochondrial dynamics (*Wang et al., 2022b*; *Liu et al., 2022*). We hypothesize that the reduction of those genes means the inhibition of mitophagy. DRP1 prevents apoptosis by promoting mitochondrial autophagy (*Pernaute et al., 2022*). Mfn2 is a pivotal mediator of mitophagy through the PINK1/Parkin signaling pathway; it facilitates Parkin recruitment from the cytosol to the depolarized mitochondria (*Uchikado, Ikeda & Ohishi, 2022*). These results indicate that the effect of CA in improving mitochondrial quality is most likely achieved by promoting mitophagy.

CA can also improve the mRNA expression of PGC-1α. The PGC-1α protein plays a key regulatory role in mitochondrial biosynthesis, which affects the energy supply for the initiation of mitochondrial autophagy (*Li, Hou & Hao, 2017*). In addition, PGC-1α is closely related to mitochondrial energy metabolism and glycolysis (*Sreekumar et al., 2022*). We are performing subsequent experiments relevant to this topic and will present the results when they are available. In our opinion, CA has positive effects on mitochondria biosynthesis, and it is likely to regulate the mitochondrial quality in multiple ways. Furthermore, there are some related genes that were not involved in this study, such as heat shock protein (HSP), voltage dependent anion channels (VDAC) and prohibitins (PHBs), which are expected to be explored in future studies. The role and impact of HSP is highly complex. Any change in the cellular dynamic equilibrium activates the synthesis of HSP, including mitochondrial stress (*Minnaar & Szasz, 2022*). The VDAC control the flux of most anionic respiratory substrates, ATP and small cations across the outer mitochondrial membrane, which is closely related to mitochondrial metabolism (*Heslop et al., 2022*). PHBs, which are evolutionarily strongly conserved proteins, are able to stabilize mitochondrial translation products (*Aboouf et al., 2021*).

In this study, we hypothesized that mitochondrial autophagy was the most critical link, because we found that CA can boost the expressions of autophagy-related proteins. Beclin-1 is an essential protein related to the formation of autophagosomes. A lack of Beclin-1 will inhibit the occurrence of autophagy (*Sun et al., 2018*). LC3-II represents the formation of autophagosomes, and it is located on the autophagosome membrane waiting for P62, then the two form a complex and finally degrade in autophagosomes (*Rogov et al., 2017*; *Liu et al., 2017*; *Yamashita & Kanki, 2017*).

Mitochondrial autophagy is interactively regulated by numerous mechanisms including the Parkin-dependent pathway, which is mainly mediated by PINK1-Parkin (*Nah, Miyamoto & Sadoshima, 2017*). PINK1 relies on the mitochondrial potential difference to transfer to the inner membrane, and is quickly degraded by related proteins in normal conditions (*Shin & Chung, 2020*). When the mitochondrial function is weakened and the level of MMP is decreased, PINK1 will accumulate on the outer membrane. Then, Parkin in the cytoplasm will be recruited and transferred to the outer membrane. Through a series of effects such as protein ubiquitination, it attracts the autophagy receptor P62, which initiates mitochondrial autophagy (*Bayne & Trempe, 2019*). In this experiment, we found that CA can up-regulate the PINK1/Parkin signaling pathway. PINK1 protein accumulated on the outer membrane can lead to autophagy through the PINK1/Parkin signaling pathway. PINK1 can also mediate MFN2 and DRP1 to regulate mitochondrial fusion and division, and assist in the occurrence of autophagy (*Chen & Dorn, 2013*).

We also confirmed the protective effects of CA on LMSCs under oxidative stress through the detection of SOD levels, ROS ratios, and apoptosis rates. SOD is a widespread metal enzyme. Its antioxidant ability stems from the catalysis of superoxide anion. SOD can catalyze the harmful superoxide anion to hydrogen peroxide, which eventually becomes water (*Sirisena et al., 2019*). In addition, ROS plays a key role in oxidative stress; the excessive production of ROS can trigger and strengthen the oxidative stress response and induce the pathological apoptosis of cells (*He et al., 2018*; *Wu et al., 2018*). In this
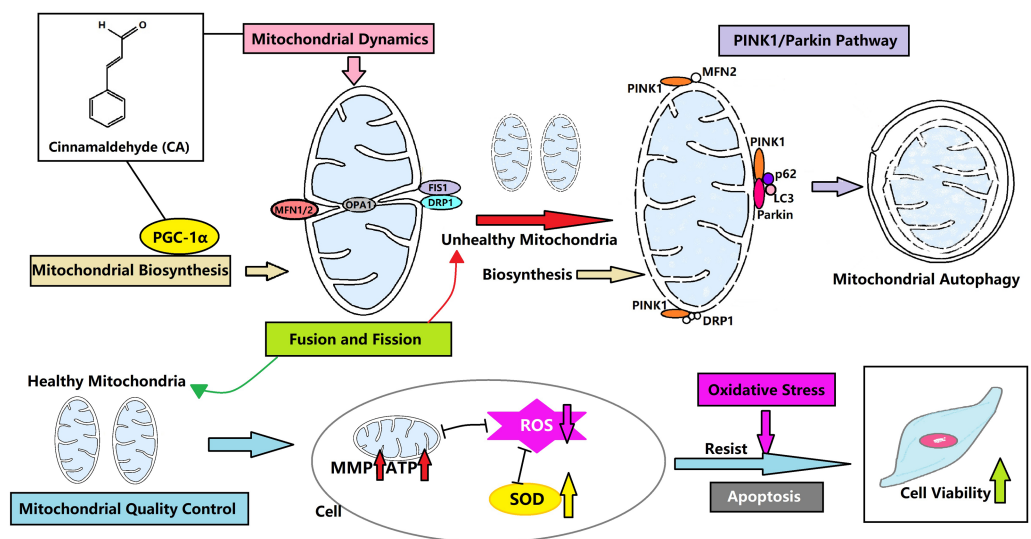
Figure 6 **CA regulates the mitochondrial quality and enhances the vitality of LMSCs.**

experiment, CA strengthened the antioxidant capacity of cells by regulating SOD and ROS levels, thus reducing apoptosis under oxidative stress. However, it is not clear whether the antioxidant capacity of CA is due to the antioxidant effect of the drug itself or the enhancement of the mitochondrial quality of cells.

CONCLUSIONS

CA can control the mitochondrial quality of LMSCs to resist oxidative stress and avoid apoptosis by regulating the dynamics, biosynthesis and autophagy of mitochondria (Fig. 6). This may be a new direction for the treatment of IPF. We hypothesize that mitochondrial autophagy is the critical link, and CA may promote autophagy through the PINK1/Parkin signaling pathway. CA is likely to regulate the mitochondrial quality in multiple ways, and our team plans to conduct animal experiments and further research in this area in the future.

Funding

The work was supported by the National Natural Science Foundation of China (No. 81560765, No. 81660780, No. 81860826), the Jiangxi University of Chinese Medicine Science and Technology Innovation Team Development Program (No. CXTD22011) and the Natural Science Foundation of Jiangxi Province (No. 20192ACBL20023, No. 20192BAB205101, No. 20212BAB216061, No. 20212BAB206061, No. 20212BAG70038). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
National Natural Science Foundation of China: 81560765, 81660780, 81860826.
Jiangxi University of Chinese Medicine Science and Technology Innovation Team Development Program: CXTD22011.
Natural Science Foundation of Jiangxi Province: 20192ACBL20023, 20192BAB205101, 20212BAB216061, 20212BAB206061, 20212BAG70038.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Shiwen Ke performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Wei Zhu conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Zhihui Lan analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Yuanbing Zhang analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Lisha Mo performed the experiments, prepared figures and/or tables, and approved the final draft.
- Guoshuang Zhu performed the experiments, prepared figures and/or tables, and approved the final draft.
- Liangji Liu conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

This study was approved by Animal Research Ethics Committee in the Jiangxi University of Traditional Chinese Medicine.

Data Availability

The data are provided in the Supplementary File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.14045#supplemental-information.

REFERENCES

Aboouf MA, Armbruster J, Thiersch M, Gassmann M, Gödecke A, Gnaiger E, Kristiansen G, Bicker A, Hankeln T, Zhu H, Gorr TA. 2021. Myoglobin, expressed in brown adipose tissue of mice, regulates the content and activity of

mitochondria and lipid droplets. *Biochimica et Biophysica Acta* **1866**(**12**):159026 DOI 10.1016/j.bbalip.2021.159026.

Absalan A, Mesbah-Namin SA, Tiraihi T, Taheri T. 2016. The effects of cinnamaldehyde and eugenol on human adipose-derived mesenchymal stem cells viability, growth and differentiation: a cheminformatics and *in vitro* study. *Biomaterials Science* **6**(**6**):643–657.

Averyanov A, Koroleva I, Konoplyannikov M, Revkova V, Lesnyak V, Kalsin V, Danilevskaya O, Nikitin A, Sotnikova A, Kotova S, Baklaushev V. 2020. First-in-human high-cumulative-dose stem cell therapy in idiopathic pulmonary fibrosis with rapid lung function decline. *Stem Cells Translational Medicine* **9**(**1**):6–16 DOI 10.1002/sctm.19-0037.

Bayne AN, Trempe JF. 2019. Mechanisms of PINK1, ubiquitin and Parkin interactions in mitochondrial quality control and beyond. *Cellular and Molecular Life Sciences* **76**(**23**):4589–4611 DOI 10.1007/s00018-019-03203-4.

Cárdenes N, Álvarez D, Sellarés J, Peng Y, Corey C, Wecht S, Nouraie SM, Shanker S, Sembrat J, Bueno M, Shiva S, Mora AL, Rojas M. 2018. Senescence of bone marrow-derived mesenchymal stem cells from patients with idiopathic pulmonary fibrosis. *Stem Cell Research & Therapy* **9**(**1**):257 DOI 10.1186/s13287-018-0970-6.

Chen Y, Dorn GN. 2013. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science* **340**(**6131**):471–475 DOI 10.1126/science.1231031.

Choi YH. 2021. Trans-cinnamaldehyde protects C2C12 myoblasts from DNA damage, mitochondrial dysfunction and apoptosis caused by oxidative stress through inhibiting ROS production. *Genes Genomics* **43**(**4**):303–312 DOI 10.1007/s13258-020-00987-9.

Doyle AA, Stephens JC. 2019. A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia* **139**:104405 DOI 10.1016/j.fitote.2019.104405.

Fergie N, Todd N, McClements L, McAuley D, O'Kane C, Krasnodembskaya A. 2019. Hypercapnic acidosis induces mitochondrial dysfunction and impairs the ability of mesenchymal stem cells to promote distal lung epithelial repair. *The FASEB Journal* **33**(**4**):5585–5598 DOI 10.1096/fj.201802056R.

Han L, Mei J, Ma J, Wang F, Gu Z, Li J, Zhang Z, Zeng Y, Lou X, Yao X, Tao N, Qin Z. 2020. Cinnamaldehyde induces endogenous apoptosis of the prostate cancer-associated fibroblasts via interfering the Glutathione-associated mitochondria function. *Medical Oncology* **37**(**10**):91 DOI 10.1007/s12032-020-01417-2.

He R, Cui M, Lin H, Zhao L, Wang J, Chen S, Shao Z. 2018. Melatonin resists oxidative stress-induced apoptosis in nucleus pulposus cells. *Life Sciences* **199**:122–130 DOI 10.1016/j.lfs.2018.03.020.

Heslop KA, Burger P, Kappler C, Solanki AK, Gooz M, Peterson YK, Mills C, Benton T, Duncan SA, Woster PM, Maldonado EN. 2022. Small molecules targeting the NADH-binding pocket of VDAC modulate mitochondrial metabolism in hepatocarcinoma cells. *Biomedicine & Pharmacotherapy* **150**:112928 DOI 10.1016/j.biopha.2022.112928.

Ikeda Y, Shirakabe A, Brady C, Zablocki D, Ohishi M, Sadoshima J. 2015. Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system. *Journal of Molecular and Cellular Cardiology* **78**:116–122 DOI 10.1016/j.yjmcc.2014.09.019.

Ismail BS, Mahmoud B, Abdel-Reheim ES, Soliman HA, Ali TM, Elesawy BH, Zaky MY. 2022. Cinnamaldehyde mitigates atherosclerosis induced by high-fat diet via modulation of hyperlipidemia, oxidative stress, and inflammation. *Oxidative Medicine and Cellular Longevity* **2022**:4464180.

Kubli DA, Gustafsson AB. 2012. Mitochondria and mitophagy: the yin and yang of cell death control. *Circulation Research* **111(9)**:1208–1221 DOI 10.1161/CIRCRESAHA.112.265819.

Li Q, Gao Z, Chen Y, Guan MX. 2017. The role of mitochondria in osteogenic, adipogenic and chondrogenic differentiation of mesenchymal stem cells. *Protein & Cell* **8(6)**:439–445 DOI 10.1007/s13238-017-0385-7.

Li PA, Hou X, Hao S. 2017. Mitochondrial biogenesis in neurodegeneration. *Journal of Neuroscience Research* **95(10)**:2025–2029 DOI 10.1002/jnr.24042.

Liu H, Dai C, Fan Y, Guo B, Ren K, Sun T, Wang W. 2017. From autophagy to mitophagy: the roles of P62 in neurodegenerative diseases. *Journal of Bioenergetics and Biomembranes* **49(5)**:413–422 DOI 10.1007/s10863-017-9727-7.

Liu M, Du F, Liu F, Wang XH. 2022. Carbon monoxide-releasing molecule-2 ameliorates postresuscitation myocardial dysfunction in rat via mitochondrial-mediated apoptosis pathway and the regulation of mitochondrial dynamics. *European Journal of Pharmacology* **927**:175038 DOI 10.1016/j.ejphar.2022.175038.

Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, Swigris JJ, Taniguchi H, Wells AU. 2017. Idiopathic pulmonary fibrosis. *Nature Reviews Disease Primers* **3**:17074 DOI 10.1038/nrdp.2017.74.

Minnaar CA, Szasz A. 2022. Forcing the antitumor effects of hsps using a modulated electric field. *Cells* **11(11)**:1838 DOI 10.3390/cells11111838.

Nah J, Miyamoto S, Sadoshima J. 2017. Mitophagy as a protective mechanism against myocardial stress. *Comprehensive Physiology* **7(4)**:1407–1424.

Onishi M, Yamano K, Sato M, Matsuda N, Okamoto K. 2021. Molecular mechanisms and physiological functions of mitophagy. *The EMBO Journal* **40(3)**:e104705.

Pernaute B, Pérez-Montero S, Nieto JMSánchez, Di Gregorio A, Lima A, Lawlor K, Bowling S, Liccardi G, Tomás A, Meier P, Sesaki H, Rutter GA, Barbaric I, Rodríguez TA. 2022. DRP1 levels determine the apoptotic threshold during embryonic differentiation through a mitophagy-dependent mechanism. *Developmental Cell* **57(11)**:1316–1330 DOI 10.1016/j.devcel.2022.04.020.

Richeldi L, Collard HR, Jones MG. 2017. Idiopathic pulmonary fibrosis. *Lancet* **389(10082)**:1941–1952 DOI 10.1016/S0140-6736(17)30866-8.

Rogov VV, Suzuki H, Marinković M, Lang V, Kato R, Kawasaki M, Buljubašić M, Šprung M, Rogova N, Wakatsuki S, Hamacher-Brady A, Dötsch V, Dikic I, Brady NR, Novak I. 2017. Phosphorylation of the mitochondrial autophagy receptor
Nix enhances its interaction with LC3 proteins. *Scientific Reports* **7**(**1**):1131 DOI 10.1038/s41598-017-01258-6.

Rovira-Llopis S, Bañuls C, Diaz-Morales N, Hernandez-Mijares A, Rocha M, Victor VM. 2017. Mitochondrial dynamics in type 2 diabetes: pathophysiological implications. *Redox Biology* **11**:637–645 DOI 10.1016/j.redox.2017.01.013.

Shin WH, Chung KC. 2020. Human telomerase reverse transcriptase positively regulates mitophagy by inhibiting the processing and cytoplasmic release of mitochondrial PINK1. *Cell Death & Disease* **11**(**6**):425 DOI 10.1038/s41419-020-2641-7.

Sirisena DMKP, Perera NCN, Godahewa GI, Kwon H, Yang H, Nam BH, Lee J. 2019. A manganese superoxide dismutase (MnSOD) from red lip mullet, Liza haematocheila: evaluation of molecular structure, immune response, and antioxidant function. *Fish and Shellfish Immunology* **84**:73–82 DOI 10.1016/j.fsi.2018.09.070.

Song M, Franco A, Fleischer JA, Zhang L, Dorn 2nd GW. 2017. Abrogating mitochondrial dynamics in mouse hearts accelerates mitochondrial senescence. *Cell Metabolism* **26**(**6**):872–883 DOI 10.1016/j.cmet.2017.09.023.

Sreekumar PG, Reddy ST, Hinton DR, Kannan R. 2022. Mechanisms of RPE senescence and potential role of αB crystallin peptide as a senolytic agent in experimental AMD. *Experimental Eye Research* **215**:108918 DOI 10.1016/j.exer.2021.108918.

Sun Y, Yao X, Zhang QJ, Zhu M, Liu ZP, Ci B, Xie Y, Carlson D, Rothermel BA, Sun Y, Levine B, Hill JA, Wolf SE, Minei JP, Zang QS. 2018. Beclin-1-dependent autophagy protects the heart during sepsis. *Circulation* **138**(**20**):2247–2262 DOI 10.1161/CIRCULATIONAHA.117.032821.

Tzouvelekis A, Toonkel R, Karampitsakos T, Medapalli K, Ninou I, Aidinis V, Bouros D, Glassberg MK. 2018. Mesenchymal stem cells for the treatment of idiopathic pulmonary fibrosis. *Frontiers in Medicine* **5**:142 DOI 10.3389/fmed.2018.00142.

Uchikado Y, Ikeda Y, Ohishi M. 2022. Current understanding of the pivotal role of mitochondrial dynamics in cardiovascular diseases and senescence. *Frontiers in Cardiovascular Medicine* **9**:905072 DOI 10.3389/fcvm.2022.905072.

Van der Bliek AM, Sedensky MM, Morgan PG. 2017. Cell biology of the mitochondrion. *Genetics* **207**(**3**):843–871 DOI 10.1534/genetics.117.300262.

Wai T, Langer T. 2016. Mitochondrial dynamics and metabolic regulation. *Trends in Endocrinology & Metabolism* **27**(**2**):105–117 DOI 10.1016/j.tem.2015.12.001.

Wang Q, Jia X, Li X, He M, Hao JN, Guan M, Mao Y, Cao Y, Dai B, Li Y. 2022a. One-pot fabrication of a polydopamine-based nanoplatform for GSH triggered trimodal ROS-amplification for cancer therapy. *Biomaterials Science* (Epub ahead of print).

Wang X, Tang Y, Xie N, Bai J, Jiang S, Zhang Y, Hou Y, Meng X. 2022b. Salidroside, a phenyl ethanol glycoside from Rhodiola crenulata, orchestrates hypoxic mitochondrial dynamics homeostasis by stimulating Sirt1/p53/Drp1 signaling. *Journal of Ethnopharmacology* **293**:115278 DOI 10.1016/j.jep.2022.115278.

Wu Z, Wang H, Fang S, Xu C. 2018. Roles of endoplasmic reticulum stress and autophagy on H_2O_2-induced oxidative stress injury in HepG2 cells. *Molecular Medicine Reports* **18**(**5**):4163–4174.

Xaubet A, Ancochea J, Molina-Molina M. 2017. Idiopathic pulmonary fibrosis. *Medicina Clinica* **148(4)**:170–175 DOI 10.1016/j.medcli.2016.11.004.

Yamashita SI, Kanki T. 2017. How autophagy eats large mitochondria: autophagosome formation coupled with mitochondrial fragmentation. *Autophagy* **13(5)**:980–981 DOI 10.1080/15548627.2017.1291113.