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Mitochondrial dysfunction induced by knockdown of mortalin is rescued by Parkin

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ABSTRACT

Mutations in the *parkin* gene are the most common cause of autosomal recessive Parkinson's disease (PD). As an E3-ubiquitin ligase, Parkin is associated with mitochondrial dynamics and mitophagy. Mortalin, a molecular chaperone, is located primarily in mitochondria, where it functions to maintain mitochondrial homeostasis and antagonize oxidative stress injury. A reduced expression level of mortalin has been observed in the affected brain regions of PD patients. Mortalin also interacts with a variety of PD-related proteins and plays an indispensible role in helping native protein refolding and importing proteins into the mitochondrial matrix. Thus, the main aims of the present study were to investigate mitochondrial dysfunction induced by knockdown of mortalin and to test whether Parkin overexpression could rescue this effect. We found that lentivirus-mediated knockdown of mortalin in HeLa cells resulted in a collapse of mitochondrial membrane potential, an abnormal accumulation of reactive oxygen species and apparent alterations in mitochondrial morphology under H_2O_2-induced stress conditions. Remarkably, Parkin overexpression rescued these mitochondrial abnormalities. In HeLa cells expressing Parkin, co-immunoprecipitation of endogenous mortalin and wild-type Parkin was detected when they were treated with carbonyl cyanide 3-chlorophenylhydrazone (CCCP). In conclusion, we indicate that the relatively decreased mortalin expression level and its impaired interaction with Parkin could affect its roles in mitochondrial function.

1. Introduction

Parkinson's disease (PD) is characterized by dopaminergic neurodegeneration in the substantia nigra pars compacta (SNpc) and by the appearance of cytoplasmic inclusions, namely Lewy bodies. PD is a highly prevalent neurodegenerative disease, yet its pathogenesis remains elusive. The accumulation of abnormal proteins and mitochondrial oxidative stress may lead to impaired or apoptotic dopaminergic neurons in PD patients. Mutations in the *parkin* gene are the most common cause of autosomal recessive PD, and may also play a role in sporadic cases of PD. Recent work on Parkin underscores the importance of mitochondrial dysfunction as a central mechanism of PD pathogenesis [1–3]. As a mitochondrially localized E3 ubiquitin–protein ligase [3], Parkin is recruited selectively to impaired mitochondria, mediates Lys27-linked polyubiquitination of voltage-dependent anion channel 1 (VDAC1) and initiates mitophagy through the autophagy–lysosome pathway [1]. Parkin is also involved in the regulation of mitochondrial morphology [4], antagonizing oxidative damage to mtDNA and activating mitochondrial self-repair mechanisms [2].

Mortalin (also known as HSPA9, GRP75 and PBP74) is a molecular chaperone 70 family member that is located primarily in mitochondria, where it functions to maintain mitochondrial homeostasis and quality control. As a central component of the mitochondrial protein import machinery, mortalin binds to nuclear-encoded proteins and drives them into the mitochondrial matrix by an ATP-dependent mechanism with the help of other co-chaperones [5]. As an anti-apoptotic protein, mortalin protects cells by inhibiting the accumulation of reactive oxygen species (ROS) during glucose deprivation [6]. Increasing evidence linking mortalin to PD include reduced expression levels in affected brain regions, mortalin mutations in PD patients and interactions of mortalin with the PD-associated proteins [7,8].

Therefore, this study primarily focuses on studying the effects of mortalin knockdown on mitochondrial homeostasis and the recruitment of Parkin to mitochondria, as well as testing whether Parkin overexpression can protect against mitochondrial dysfunction induced by knockdown of mortalin under H_2O_2-induced oxidative stress.

2. Materials and methods

2.1. Plasmid construction

Parkin variants (T240R/R275W/R1 domain del) were fusion PCR generated and subcloned into pcDNA3.1 (−) (Invitrogen, Carlsbad,

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Table 1
Specific primers used for cloning and mutagenesis.

Primer name	Template (5′–3′)	Application
Parkin Forward	CCGGAATTCGCCACCATGATAGTGTTTGTCAGGTTC	Cloning (pcDNA3.1 and pEGFP)
Parkin Reverse	CGCGGATCCCACGTCGAACCAGTGGTCC	Cloning (pcDNA3.1)
Parkin Reverse (pEGFP)	CGCGGATCCCCACGTCGAACCAGTGGTCC	Cloning (pEGFP)
T240R	ATCACTTGCATTAGGTGCACAGACG	Cloning (mutagenesis)
R275W	CAAGACTCAATGATTGGCAGTTTGTTCAC	Cloning (mutagenesis)
R1 Del Reverse	CCCAACTCCTTGATTAAAGAG	Cloning (mutagenesis)
R1 Del overlap Forward	CTCTTTAATCAAGGAGTTGGGGCAAGTGATGTTCCGACTATT	Cloning (mutagenesis)

CA) or pEGFP-N2 (Clontech, Palo Alto, CA). The pLKO.1-TRC, pCMV-dR8.2 and pCMV-VSVG vectors were purchased from Sigma–Aldrich (Sigma–Aldrich, St. Louis, MO). All constructs were verified by sequencing. Primers used in this experiment were listed below (Table 1).

2.2. Cell culture and transfection

HeLa and HEK293T cells were maintained at 37 °C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; Invitrogen), supplemented with 10% (vol/vol) fetal bovine serum (FBS; BioWest, Caille, France). Lipofectamine2000 (Invitrogen) or PEI (Sigma–Aldrich) were used to transfect cells following the manufacturer's instructions or as described previously [9].

2.3. Lentivirus-mediated RNA interference

Lentivirus production was done as previously described [10]. HeLa cells were infected with a lentivirus expressing shRNA targeting the CGTGCTCAATTTGAAGGGATT sequence of mortalin. This sequence was checked against the BLAST database to confirm specificity. A scrambled shRNA sequence was used as control. Infected cells were selected in puromycin (Sigma); polyclonal cell populations were then tested for decreased mortalin expression levels.

2.4. Quantitative real-time PCR analysis

For measuring mtDNA copy number, total cellular DNA was isolated with E.N.Z.A genome DNA kit (Omega Bio-tek, Doraville, GA). Quantitative real-time PCR analysis of the mtDNA copy number was performed as previously described [11]. To measure target mRNA expression levels, total RNA was prepared from cells using RNAiso Plus (TaKaRa, Dalian, China). First-strand cDNA was synthesized using the RevertAid first-strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania). PCR products were performed by real-time PCR with Ultra-Fast SYBRGreen QPCR Master Mix (Agilent Technologies, Palo Alto, CA) and were analyzed in real-time with the MiniOpticon detection system (Bio-Rad Laboratories, Hercules, CA). The data were analyzed with the CFX Manager v1.5 software program using $\Delta\Delta C_t$ method. The PCR protocol was: 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 30 s. Each sample was assayed in triplicate, and three independent experiments were performed. The primers used in this experiment are listed below (Table 2).

2.5. Mitochondrial and cytosolic fractionation

Isolation of mitochondria and cytosolic fraction from HeLa cells was done as previously described [12].

2.6. Co-immunoprecipitation (CO-IP) assays

For CO-IP assays, HeLa cells were transiently transfected with the indicated plasmids. Cells were harvested and immediately lysed in a NP-40 lysis buffer (50 mM Tris, pH 7.5; 150 mM NaCl; 0.1% NP-40; 5 mM EDTA; 60 mM β-glycerophosphate; 1 mM sodium orthovanadate; 20 mM NaF; 10 μg/ml aprotinin, 10 μg/ml leupeptin; 1 mM PMSF). The supernatant was collected and mixed with 40 μl Protein A + G plus-agarose beads and various antibodies at 4 °C for 6 h with constant agitation. Following four washes with NP-40 lysis buffer, samples were denatured in 2× Laemmli sample buffer, separated by SDS–PAGE, transferred to a 0.45-μm PVDF membrane (Millipore) and subjected to immunoblot analysis.

Table 2
Specific primers used for real-time PCR.

Primer name	Template (5′–3′)	Application
Mortalin Forward	AGCTGGAATGGCCTTAGTCAT	Reverse transcription qRT-PCR
Mortalin Reverse	CAGGAGTTGGTAGTACCCAAATC	Reverse transcription qRT-PCR
18S Forward	CGGCTACCACATCCAAGGAA	Reverse transcription qRT-PCR
18S Reverse	GCTGGAATTACCGCGGCT	Reverse transcription qRT-PCR
TK2 Forward	TCCTGCAGATGCCACTTTGA	qPCR (mtDNA copy number)
TK2 Reverse	CCCCAAGTCTGAAGAAAACG	qPCR (mtDNA copy number)
mtDNA-D loop Forward	CATCTGGTTCCTACTTCAGGG	qPCR (mtDNA copy number)
mtDNA-D loop Reverse	TGAGTGGTTAATAGGGTGATAGA	qPCR (mtDNA copy number)

2.7. Immunofluorescence

For Immunofluorescence (IF) analysis, HeLa cells were plated on coverslips in 6-well plates and transfected with the indicated constructs for 36 h. Cells were washed with phosphate buffered saline (PBS), fixed in 4% paraformaldehyde in PBS for 30 min at room temperature (RT), washed three times with PBS, permeabilized with 0.5% Triton X-100 in PBS for 5 min at RT and blocked with 10% FBS in PBS 1 h at 4 °C. Cells were incubated for 2 h at RT with antibodies diluted in 5% FBS in PBS followed by incubation with secondary antibodies for 1 h. Nuclei were stained with Hoechst33258 (Sigma). Coverslips were mounted onto microscope slides using fluorescence mounting medium. Images were obtained with a DM2000 fluorescence microscope (Leica Microsystems, Wetzlar, Germany) equipped with a Leica DFC420 camera and analyzed by ImageJ 1.44j (Wayne Rasband, NIH, Bethesda, MD).

2.8. Quantification of mitochondrial morphology

Mitochondrial morphology was measured as previously described [13]. The mitochondrial morphology was investigated by using a fluorescence microscope equipped with a Leica DFC420 camera. By means of ImageJ 1.44, images were optimized by adjusting the contrast to span the entire available gray range from 0 to 255 and converted to 8-bit binary format. A $7 * 7$ "top-hat" spatial filter with threshold was then applied to define the mitochondrion area and outline. Aspect ratio (AR, which is the ratio

between the major and minor axes of an ellipse equivalent to the object) was analyzed using ImageJ.

2.9. Annexin V/propidium iodide (PI) staining

Apoptosis was measured using an Annexin V-FITC/PI apoptosis detection kit (BD Pharmingen, San Diego, CA) according to the manufacturer's instructions.

2.10. Intracellular ROS and MMP detection

MMP was measured using Mitotracker Red CMXros (MTR; Invitrogen) and MitoTracker Green FM (MTG; Invitrogen) as previously described [14]. Intracellular ROS were measured using 2′,7′-dichlorofluorescein-dictate probe (DCFH-DA; Invitrogen, Carlsbad, CA). After incubation with H_2O_2, cells were loaded with 10 μM DCFH-DA for 30 min at 37 °C in the dark. Cells were then analyzed on a Spectramax M5 microplate reader (Molecular Devices, San Francisco, CA). The experiment was performed in triplicate.

3. Results

3.1. The CCCP induced interaction of endogenous mortalin with Parkin in vivo

To determine whether CCCP can induce the interaction of endogenous mortalin with Parkin in vivo, we first examined the subcellular localization of Parkin and mortalin. Because of undetectable Parkin levels in HeLa cells, the cells were transiently transfected with pEGFP (for immunofluorescence analysis) or pcDNA 3.1(−) (for subcellular fractionation analysis) vector encoding wild-type (WT) Parkin and treated with 10 μM CCCP for 1 h. The results of immunofluorescence confirmed that mortalin is majorly localized to mitochondria, while Parkin distributes throughout the cytosol under normal conditions. Upon exposure to 10 μM CCCP for 1 h, we investigated the recruitment of Parkin to mitochondria (Fig. 1A). The results of subcellular fractionation also indicated that mortalin and Parkin can express in mitochondria (Fig. 1A, right panel). The C-terminal of Parkin contains two RING-finger motifs

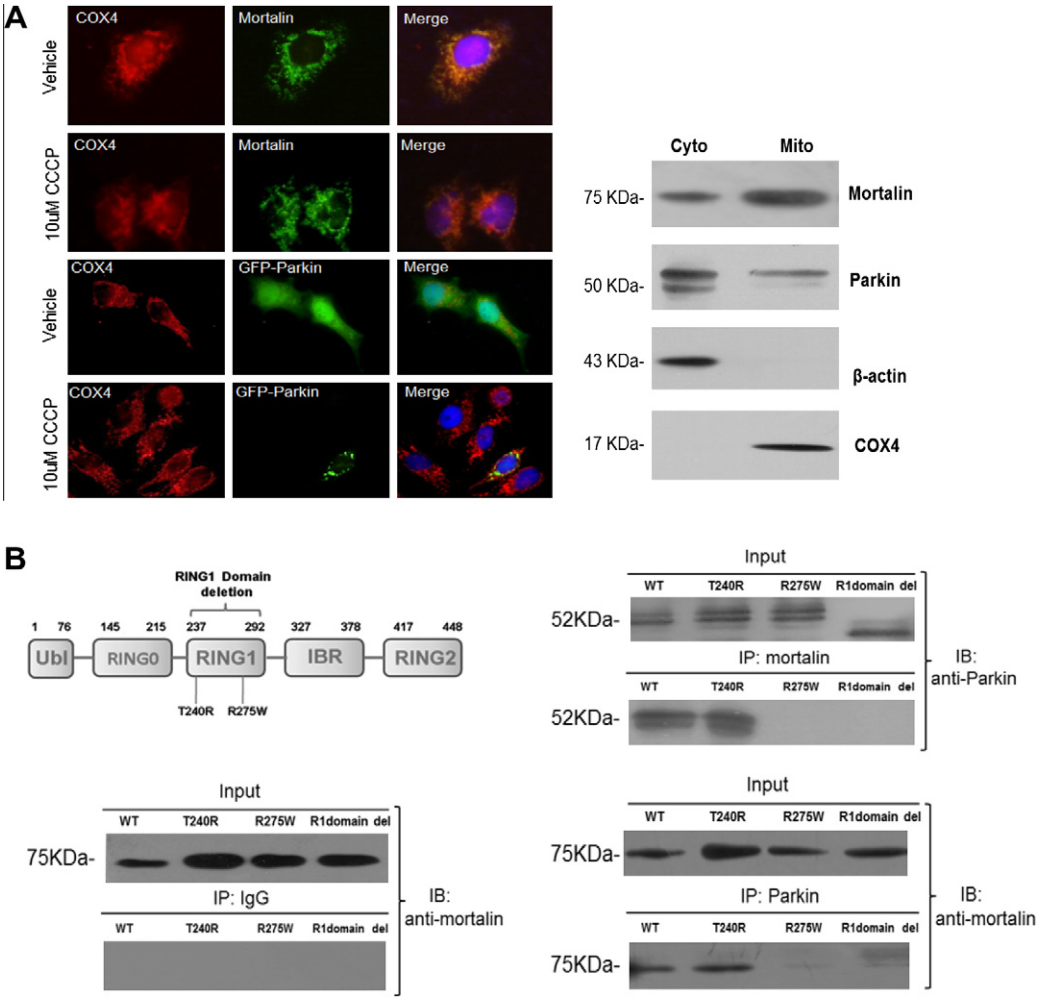

Fig. 1. The CCCP induced interaction of endogenous mortalin with Parkin in vivo. (A) Subcellular location of mortalin and Parkin: immunofluorescence analyses of subcellular location of mortalin and Parkin. HeLa cells were transiently transfected pEGFP-vector encoded WT Parkin for 36 h and treated with 10 μM CCCP for 1 h. Mitochondria were stained red by MTR or immunostained with COX-4 antibody, nuclei were labeled blue with Hochest 33258. Fractionation analysis was conducted by isolation mitochondria from HeLa cells transiently transfected with pcDNA3.1 (−) vector encoding WT Parkin then subjected to immunoblot analysis. (B) Schematic representation of vectors encoding Parkin and the CCCP induced interaction of endogenous mortalin with Parkin. Schematic representation of vectors encoding Parkin. UBL, ubiquitin-like domain; RING, RING-finger motifs; IBR, in-between RING-finger domain. R1 domain deletion meant the nucleotides encoding 238–292 amino acids of Parkin were deleted by fusion PCR. Locations of the different mutations used in this study were indicated on the domain structure. For CO-IP, HeLa cells were transiently transfected with pcDNA3.1 (−) vectors encoding WT or MT Parkin for 36 h and treated with 10 μM CCCP for 1 h. NP-40-solubilized lysates from HeLa cells were immunoprecipitation with anti-Parkin, IgG or mortalin antibodies followed by immunoblot analysis with the indicated antibodies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

(R1 and R2), and previous studies identified that R1 domain-related mutations in Parkin impair its subcellular localization [13]. We therefore introduced R1 domain-related mutations using fusion PCR. Locations of the different mutations used in this study were indicated on the domain structure (Fig. 1B). To investigate the CCCP induced interactions of endogenous mortalin with WT or mutant type (MT) Parkin, we performed CO-IP assays using lysates from transiently transfected HeLa cells expressing WT or MT Parkin, and indicated that the R275W and R1 domain deletion (but not the T240R mutation) impaired the ability of Parkin to interact with endogenous mortalin (Fig. 1B).

3.2. Lentivirus-mediated knockdown of mortalin in HeLa cells

Because mortalin is relatively reduced in affected brain regions of PD patients [8], HeLa cells were infected with a lentivirus expressing shRNA targeting the sequence of mortalin. After formation of puromycin-resistant clones, real-time PCR and Western blot analysis were performed. As shown in Fig. 2A, the mortalin mRNA expression level in KD group was 0.25 ± 0.09-fold compared with the control ($^{*}P < 0.05$). Immunoblot analysis also revealed that Lentivirus-mediated knockdown led to notable suppression of mortalin expression (Fig. 2B, bottom panel). We found that lentivirus-mediated RNA interference exerted a powerful and unitary knockdown of mortalin expression, while no knock-out effect was observed following scrambled shRNA infection (data not shown).

3.3. The effects of knockdown of mortalin on mitochondrial morphology, apoptosis and Parkin-mitochondrial translocation

To determine the effects of knockdown of mortalin on the mitochondrial morphology, HeLa cells were stained with MTG, a mitochondrion-specific fluorescent probe, and visualized by fluorescence microscopy. We found obvious truncated and fragmented mitochondria in mortalin KD group under H_2O_2-induced stress conditions (Fig. 3A, left panel). Aspect ratio (AR), one of the important morphological parameters in mitochondria was quantified using ImageJ software. No significant alterations of mitochondrial length (AR) were observed in control and mortalin KD HeLa cells under basal conditions. However, upon exposure to 600 μM H_2O_2 for 1 h, the mortalin KD group showed a significant decrease in elongation ($^{*}P < 0.05$; Fig. 3A, right panel).

To determine the effects of knockdown of mortalin on apoptosis, HeLa cells were treated with vehicle or 600 μM H_2O_2 for 1 h and the rates of apoptosis were assessed by Annexin V/PI staining followed by flow cytometry analysis. There were no differences between the KD and control groups under basal conditions. However after treatment with 600 μM H_2O_2 for 1 h, the early and late apoptotic rate significantly increased (by nearly 15%) in the KD groups compared with control cells ($^{*}P < 0.05$; Fig. 3B).

As a central component of the mitochondrial protein import machinery, mortalin binds to nuclear-encoded proteins and drives them into the mitochondrial matrix. To test the effects of knockdown of mortalin on recruitment of Parkin to mitochondria, HeLa cells were transfected with a plasmid encoding GFP-tagged WT Parkin for 36 h and treated with vehicle or 10 μm CCCP for 1 h. The colocalization of GFP–Parkin with mitochondria in control and mortalin KD HeLa cells were examined by fluorescence microscopy. We found that partial knockdown of mortalin in HeLa cells did not abrogate the recruitment of Parkin to mitochondria upon addition of CCCP ($^{*}P > 0.05$; Fig. 3C).

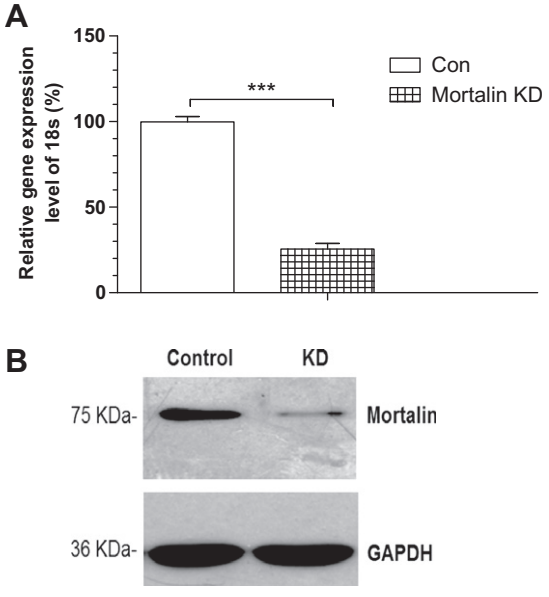

Fig. 2. Lentivirus-mediated knockdown of mortalin in HeLa cells. (A) Real-time PCR analysis. HeLa cells were infected with lentivirus shRNA and selected with 2.5 μg/ml puromycin until resistant clones formed. The mRNA expression level of mortalin in knockdown group were significantly decreased compared to control ($n = 3$, $^{***}P < 0.01$). Data is normalized as percentage of control group and represent as means ± SEM. (B) Immunoblot analysis. Mortalin protein in knockdown group decreased remarkably compared to control. GAPDH was used as an internal control to normalize protein loading. The representative result of three experiments was shown.

3.4. Parkin overexpression rescues the mitochondrial dysfunction induced by knockdown of mortalin

To determine the role of mortalin and Parkin in H_2O_2-induced mitochondrial dysfunction, MMP, ROS and mtDNA copy numbers were monitored. Intracellular ROS levels were measured with DCFH-DA. Under normal conditions, the basal levels of ROS accumulation were nearly the same; Upon exposure to 600 μM H_2O_2 for 1 h, we observed mortalin KD group had about a 50% increase in levels of DCF fluorescence ($^{***}P < 0.01$; Fig. 4A), and overexpression of WT Parkin in mortalin KD group markedly prevented ROS accumulation induced by H_2O_2, but MT Parkin failed.

MMP levels were measured with MTR and MTG. MTG stains all mitochondria while MTR stains only high MMP mitochondria. So the MTR/MTG ratio can therefore be used to monitor changes in MMP. Under normal conditions, no significant differences were found between the four groups regarding changes in the MTR/MTG ratio; however, upon exposure to 600 μM H_2O_2 for 1 h, MMP in the mortalin KD groups was reduced by 31.43 ± 5.9% compared with the control group. Overexpressing WT, but not R1del Parkin rescued the decrease of MMP ($^{***}P < 0.01$; Fig. 4B).

mtDNA synthesis is a limiting factor in the mitochondrial remodeling process and essential for normal cellular function [15]. We observed that knockdown of mortalin induced a 40.2 ± 3.8% reduction in mtDNA copy numbers under normal conditions, and overexpressing Parkin rescued this reduction ($^{***}P < 0.05$; Fig. 4C).

4. Discussion

Our data show that mortalin knockdown induces an unexpected collapse of MMP, abnormal accumulation of ROS and a significant decrease of mtDNA copy numbers under H_2O_2-induced oxidative stress conditions. Moreover, mortalin interacts with the PD-related protein Parkin, and Parkin overexpression notably rescues the mitochondrial dysfunction that is induced by knockdown of mortalin.

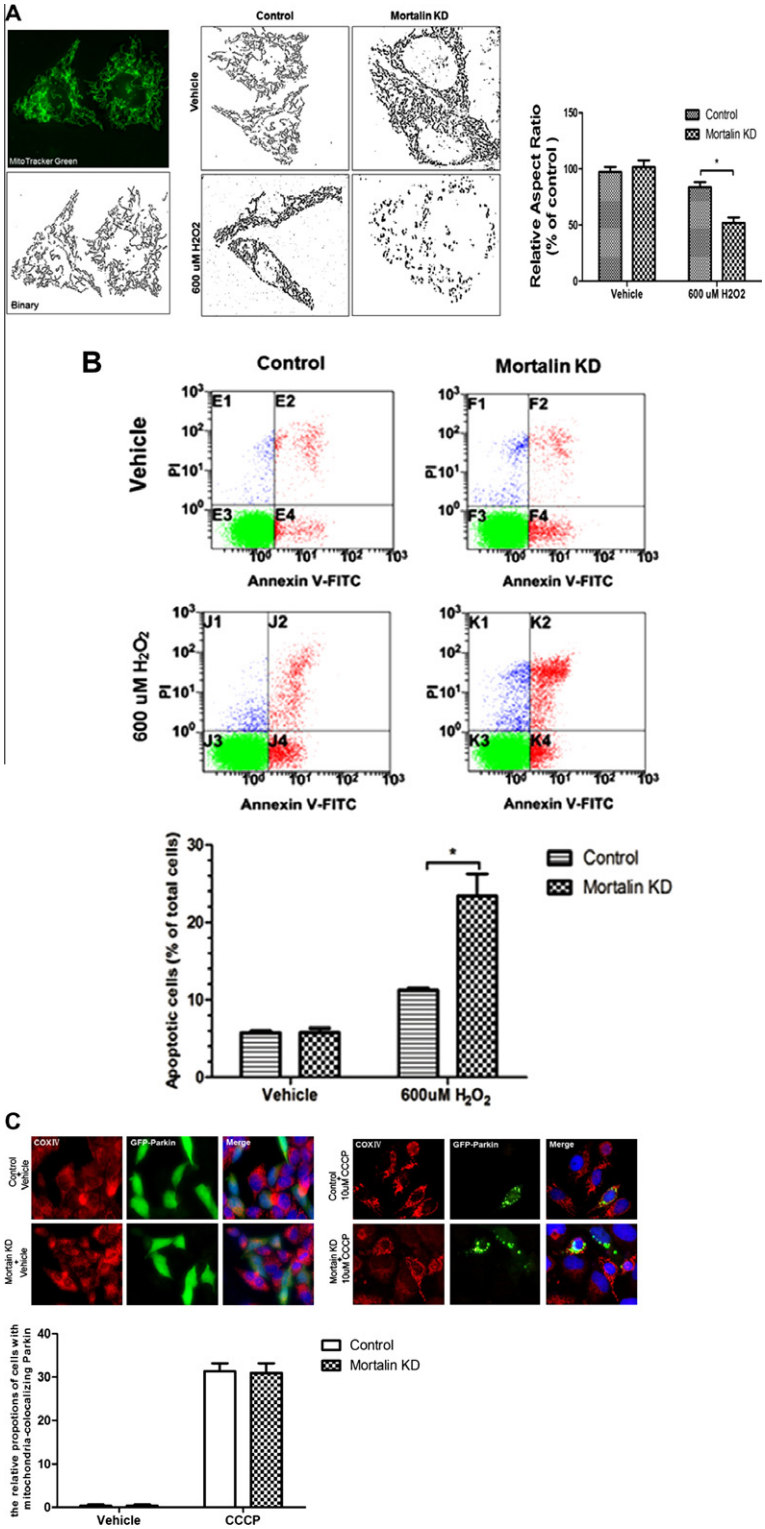

Fig. 3. The effects of knockdown of mortalin on mitochondrial morphology, apoptosis and Parkin-mitochondrial translocation. (A) Quantification of mitochondrial morphological parameters. Mitochondria from control and mortalin KD HeLa cells were labeled by a specific mitochondrial dye: MitoTracker Green (MTG) for 30 min at 37 °C and viewed with fluorescence microscope. AR, as a mitochondrial morphological parameter, was quantified by ImageJ software. Images from total 20 individual cells were analyzed through three independent experiences and data were presented as Mean ± SEM. $^{*}P < 0.05$ versus H_2O_2-treated control. (B) The rates of apoptosis in control and mortalin KD HeLa cells were analyzed by flow cytometry. Control or mortalin KD HeLa cells were treated with H_2O_2 and the rate of apoptosis were confirmed by flow cytometry with Annexin V/propidium iodide (PI) double staining. Apoptosis degree was shown as apoptosis index evaluated by counting the percentage of apoptotic cells. The degree of apoptotic cells was analyzed through three independent experiments and data were presented as Mean ± SEM, $^{*}P < 0.05$ versus H_2O_2-treated control. (C) Quantification of Parkin-mitochondrial translocation in control and mortalin KD HeLa cells. HeLa cells were transiently transfected with GFP tagged WT Parkin vector (green) for 36 h, and treated with vehicle or 10 μM CCCP for 1 h then immunostained with anti COX-4 (Red) to label mitochondria. Nuclei were stained blue with Hochest 33258. At least 100 cells per condition and three independent experiments were performed. Data were scored and presented as means ± SEM ($n = 3$, $P > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

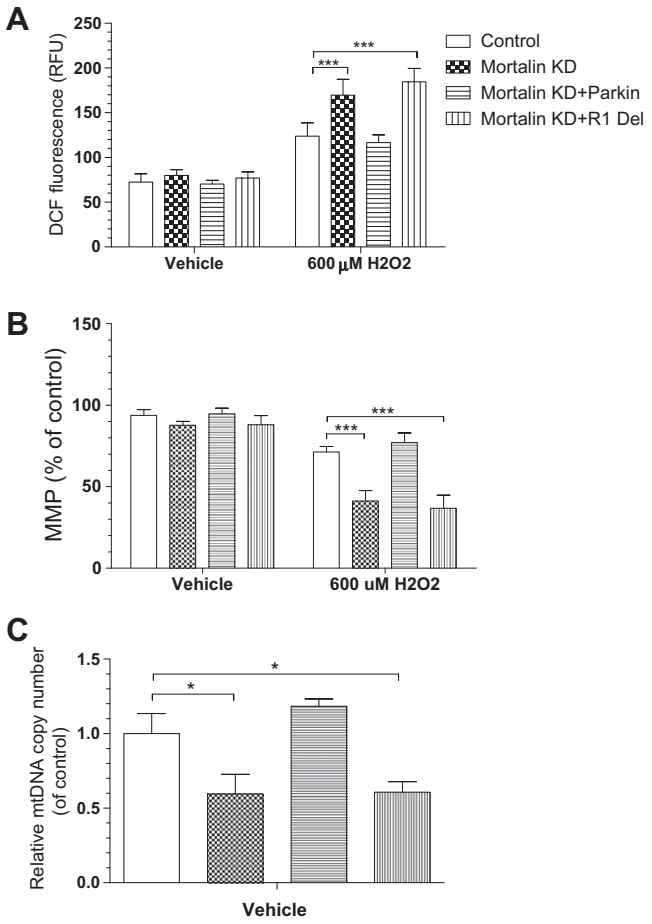

Fig. 4. Parkin overexpression rescues the mitochondrial dysfunction induced by knockdown of mortalin. (A) Parkin overexpression mitigated ROS accumulation. Mortalin KD HeLa cells were transfected with WT Parkin, R1 Del Parkin as indicated. After exposure to 600 μM H_2O_2 for 1 h, cells were incubated with DCFH-DA at 37 °C for 20 min. The intracellular ROS levels were assayed by measuring the fluorescence intensity of DCF. Data were represented as means ± SEM for three independent experiments. $^{***}P < 0.01$ versus H_2O_2-treated control. (B) Parkin overexpression inhibited the dissipation of MMP. The indicated HeLa cells were treated as described and the MMP levels were determined by using two mitochondria specific fluorescent probes: MTR and MTG. MMP in each group was calculated as the fluorescence ratio of MTR/MTG. Results were expressed as percentage of vehicle-treated control and represented as means ± SEM for three independent experiments. $^{***}P < 0.01$ versus H_2O_2-treated control. (C) Parkin overexpression enhanced the mtDNA copy numbers. Mitochondrial DNA copy numbers were determined by quantitative real-time PCR using primers against the mitochondrial displacement loop (D-loop) and the nuclear single copy gene: thymidine kinase 2 (TK2), which is as an internal standard. The amounts of mtDNA were normalized to the internal control. $^{*}P < 0.05$ versus control group, values are means ± SEM, $n = 9$.

4.1. Oxidative damage with mitochondrial homeostasis

Mitochondria are vulnerable and at high risk for oxidative damage, as they contain multiple electron carriers. Excess ROS can attack proteins, lipids and other small substances and induce membrane lipid peroxidation and cellular redox status disruption. ROS is also a causal step in apoptosis and a key element in the pathophysiology of PD [3]. Evidences have reported that deficiency of Parkin in *Drosophila* [16] and mice [17] disturbs mitochondrial function, and patients with Parkin mutations show a selective decrease in leukocyte complex-I activity [18]. Due to its function in the mitochondrial matrix, mortalin also involves in the maintenance of mitochondrial homeostasis and prevents the accumulation of redundant ROS in mitochondria. Although these reports demonstrate that mortalin and Parkin provides a protective effect against oxidative damage, their detail roles in relation to PD needs to be addressed. In this study, we found upon exposure to H_2O_2, the oxidative stress levels in the mortalin KD group increased significantly. Furthermore, Parkin overexpression rescued the mitochondrial dysfunction that was induced by mortalin KD. Our results differ somewhat from a recent study that found under normal conditions knockdown of endogenous mortalin caused a significant increase of intramitochondrial ROS production compared with control siRNA-treated HEK293 cells [7]. One possible explanation for this discrepancy may be the difference in down-regulation methods, knockdown efficiency and ROS detection methods used in the different cell types. Our results indicate that blocking of endogenous mortalin expression exacerbates susceptibility to proapoptotic insults in HeLa cells and confirms that mortalin and Parkin plays a protective role against oxidative damage.

Despite we found that knockdown of mortalin expression in HeLa cells had no obvious effect on Parkin mitochondrial translocation, the interaction between mortalin and Parkin was verified by CO-IP. Because of their distinct subcellular localizations, it seems that oxidative stress may be a triggering event in the recruitment of Parkin to mitochondria, and induced pathological interactions between mortalin and Parkin. Because H_2O_2-induced oxidative stress can result in Parkin-mitochondrial translocation [19], initiates mitophagy of the damaged mitochondria and prevents further apoptosis. Thus, an impaired interaction between mortalin and Parkin might interfere with the protective role of Parkin.

4.2. Mitochondrial dynamics with PD

As a dynamic organelle, the mitochondrion constantly experiences fission, fusion, proliferation and degradation to maintain cellular homeostasis and biogenesis. Mitochondria have the task of maintaining a balance between spheres (fission) and networks (fusion) at various stages of the cell cycle, and perturbations of this balance can induce permeabilization of the mitochondrial outer membrane and subsequently trigger apoptosis. Fibroblasts from patients with Parkin mutation have shown remarkable abnormalities in mitochondrial morphological changes [3]. Mortalin is also involved in PD-related abnormal mitochondrial morphology; for example, decreased mortalin expression levels in the mitochondrial fraction and altered mitochondrial morphology induced by mortalin mutations in PD patient fibroblasts have been observed. Our results indicate that under H_2O_2-induced stress conditions, knockdown of mortalin induces disintegration of mitochondrial connectivity and low-grade branching of mitochondria, as shown by a decrease in mitochondrial aspect ratio. Our results emphasize the crucial role of mortalin in maintaining mitochondrial morphology and dynamics, given its principal location and function in the mitochondrial matrix.

An open question that merits future investigation is how mortalin is involved in controlling mitochondrial morphology. To the best of our knowledge, mitochondrial fission and fusion are important for maintaining mitochondrial integrity [20]. Therefore, we speculate that mortalin deficiency (caused by either mutations or decreased expression levels) in affected brain regions may perturb cellular homeostasis, induce mitochondrial morphological alterations and trigger subsequent apoptosis. Mitochondrial fission and fusion are complicated processes that involve separate reactions in the mitochondrial membranes. Mitochondrial fission is controlled by Drp1 (dynamin related protein 1), whereas fusion is controlled by Opa1 (optic atrophy 1), Mfn1 and 2 (mitofusin 1 and 2). Thus, whether mortalin interacts with fusion- and/or fission-related proteins and affects their mitochondrial location or translocation should be investigated.

Acknowledgments

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