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Parkin in early stage LPS-stimulated BV-2 cells regulates pro-inflammatory response and mitochondrial quality via mitophagy

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ABSTRACT

Numerous literature related to the role of Parkin and mitophagy focuses on neurons, but not microglial cells, responsible for most inflammatory responses in the central nervous system. Here, we first observed that Parkin expression in BV-2 microglial cells increased up to 6 hours (early stage) after LPS stimulation and decreased after that. Overexpression Parkin substantially suppressed the pro-inflammatory response and control of pro-inflammatory mediators is through regulation of MAPK and NF-κB signal in BV-2 cells. Therefore, these results demonstrate that early LPS-induced Parkin controls the inflammatory response by modulating MAPK and retains mitochondrial quality through mitophagy in microglial cells.

1. Introduction

Microglia, the resident innate immune cells of the central nervous system (CNS), are highly important components in early control of infections and for recruitment of cells of the adaptive immune system required for pathogen clearance (Nimmerjahn et al., 2005; Ransohoff and Brown, 2012). Furthermore, microglia are versatile and multifunctional cells with pivotal functions in cell survival as immune surveillance cells for maintaining brain homeostasis (Casano and Peri, 2015; Prinz and Priller, 2014).

Activated microglia in response to several stimuli such as lipopolysaccharide(LPS) express genes associated with inflammation, including pro-inflammatory cytokines/chemokines, growth factors, and free radicals (Block and Hong, 2007; Lull and Block, 2010) and also produce these substances (von Bernhardi et al., 2015). Excessive microglial activation (or unregulated microglial activation) is associated with neurodegenerative diseases. In particular, reactive oxygen species (ROS) generation by microglia is considered a major cause of neuronal dysfunction, damage, and death (Gao et al., 2012) through direct oxidative damage or disruption of neuronal redox signaling (Rojo et al., 2014). Appropriate regulation of microglial activation might therefore be key in protecting the CNS from inflammatory responses (Dello Russo et al., 2004). Additionally, activated microglia are implicated in qualitative variation in mitochondria (Banati et al., 2004).

Mitochondria are highly reserved and dynamic eukaryotic organelles involved in various functions including amino acid production (Harper et al., 1984), fatty acid synthesis (Kastaniotis et al., 2017), and iron-sulfur protein biogenesis (Stehling et al., 2014). In addition, mitochondria buffer calcium and are a signaling center for innate immunity and cell death (West et al., 2011; Zavodnik, 2016).In particular, ATP synthesis is the most important function of mitochondria, and reactive oxygen species (ROS) are generated as a byproduct via the electron transfer chain (ETC) (Murphy, 2009).Therefore, mitochondria are a prominent source of ROS; adequate control of ROS generation is essential to regulate cell signaling (Czarna and Jarmuszkiewicz, 2006). However, dysregulation of ROS can cause mitochondrial malfunction, especially excessive activation of microglia (Gao et al., 2012; Rojo et al., 2014). Thus, mitochondria need specific conditions, to prepare for quality control and modulation of microglial activation.

Mitogen-activated protein kinases (MAPKs), well-known Ser/Thr protein kinases, are activated through phosphorylation and convert extracellular stimuli into cellular responses to mediate many cellular activities, such as cell proliferation, survival, and death (Cargnello and Roux, 2011; Kim and Choi, 2010). Accumulated evidence showed that

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MAPK signaling is deeply involved in the inflammatory response (Bamford et al., 2007; Kaminska, 2005; Olajide et al., 2013), but little emphasis was laid on the interplay between mitophagy and MAPK in activated microglial cells.

Mitophagy is a form of selective autophagy that degrades impaired mitochondria. Generally, mitophagy is regulated by the proteins parkin and PTEN-induced putative kinase protein 1 (PINK1) (Durcan and Fon, 2015; Eiyama and Okamoto, 2015). Parkin is an E3 ubiquitin ligase expressed in several tissues including the brain, heart, and muscles and it polyubiquitinates its substrate through ubiquitination of different K linkages to induce various cellular signals. For example, K48-linked chains induce target proteins for destruction by the 26S proteasome (Shimura et al., 2000), whereas K63-linked ubiquitination is involved in various cellular signaling pathways such as autophagy (Komander and Rape, 2012). Most parkin is located in the cytosol under physiological conditions. In response to stress, such as mitochondrial depolarization, parkin is translocated from the cytosol to the mitochondria by PINK1 and mediates selective autophagic elimination of damaged mitochondria (Seirafi et al., 2015). Regulation of parkin expression and the amount of translocated parkin are critical for modulation of mitophagy. In addition, parkin has important functions with regard to mitochondrial function and morphology (Mortiboys et al., 2008; Muftuoglu et al., 2004). Hence, parkin should be recruited to impaired mitochondria at the appropriate time to mediate mitophagy. PINK1, another key component for mitophagy, is the protein kinase that induces parkin-mediated mitophagy by recruiting parkin (Seirafi et al., 2015). Parkin and PINK1 are interactive proteins essential for mitophagy to maintain mitochondrial homeostasis. Microglia are important components in the brain that induce mitophagy for removal of impaired mitochondria. Previous studies suggested that parkin deficiency evokes mitochondrial dysregulation and sensitizes oxidative damage that cannot maintain mitochondrial homeostasis (Palacino et al., 2004). Furthermore, recent research showed has shown that parkin deficiency disrupts age-dependent mitophagy that should be increased with aging (Cornelissen et al., 2018). However, the effect of overexpression of parkin in retaining mitochondrial quality has not yet been studied.

In this study, we hypothesized that overexpressed parkin may suppress pro-inflammatory cytokines and rescue impaired mitochondria via up-regulated mitophagy. Therefore, we determined whether pro-inflammatory mediators and cytokines were regulated by increased mitophagy during parkin overexpression in BV-2 microglial cells, and examined whether this overexpression affects regulation of mitochondrial quality.

2. Materials and methods

2.1. Cell culture and treatment

BV-2 murine microglial cells were immortalized by infection with the v-raf/myc recombinant retrovirus, which was kindly provided by Dr. Jau-Shyong Hong (NIEHS, NC, USA). Cells were cultured in Dulbecco's modified Eagle medium (DMEM; Welgene, Daegu, Korea) with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Welgene) and incubated at 37 °C in a humidified 5% CO_2 incubator (SANYO, Osaka, Japan). Exponentially growing BV-2 cells were stimulated with 1 μg/mL LPS from *Escherichia coli* serotype 026:B6 (Sigma, MO, USA) for 12 hr.

2.2. Generation of lentivirus and stable cell line

The pDsRed2-Mito Plasmid and pEGFP-parkin WT plasmid were purchased from Clontech (Mountain View, CA) and Addgene (Water town, MA, USA). A previous paper described the establishment of the lentivirus (Kim et al., 2010; Park et al., 2016). Stable cell lines with parkin overexpression were generated by infection of BV-2 cells with the lentivirus (MOI = 5) and 8 μg/mL polybrene. Parkin-transduced BV-2 cells were incubated for 72 hr and then selected with 8 μg/mL blasticidin (Sigma) for 1 week.

2.3. Quantitative real-time polymerase chain reaction

TRI-Reagent (Invitrogen, CA, USA) was used for isolation of total RNA from BV-2 and parkin overexpressing BV-2 according to the manufacturer's instructions. The concentration of isolated total RNA was calculated from absorbance at 260 nm using a spectrophotometer (ACTgene, USA), and the purity was verified by an optical density absorption ratio (260 nm/280 nm) between 1.8 and 2.0. To synthesis of Complementary DNA (cDNA), oligo dT were attached to RNA (1 μg) at 70 °C for 10 min. Then, by using a reverse transcription premix (Bioneer, Korea), RNA annealed with oligo dT were reverse-transcribed into cDNA by performing with activation of polymerase at 42 °C for 1 hr, inactivation of additional action of polymerase at 95 °C for 5 min, and stabilization at 15 °C for 5 min. All cDNA samples used as template in the subsequent quantitative PCR (qRT-PCR) analyses were stored at −20 °C. To quantify the mRNA levels, we performed real-time PCR using the SYBR Fast quantitative PCR (qPCR) Mix (Takara Bio, Kyoto, Japan). The final reaction volume was 20 μL, including 2 λ of cDNA, primers, and 10 μL of the SYBR green master mix. The qRT-PCR assays were run on a Step OnePlus Real-Time PCR machine (Applied Biosystems, CA, USA). Quantification was conducted using the comparative ΔCt method. The following primers were used for qRT-PCR amplification: 5'-Parkin, 5′- TCTTCCAGTGTAACCACCGTC -3′; 3′- Parkin, 5′- GGCAGGGAGTAGCCAAGTT -3′; 5′- TNF-α, 5′- CCTGTAG CCCACGTCGTAGC -3′; 3′- TNF-α, 5′- AGCAATGACTCCAAAGTAGACC -3′; 5′- IL-1β, 5′- ACTGTTTCTAATGCCTTCCC -3′; 3'-IL-1β, 5′- TGGTTTCTTG TGACCCTGA -3′; 5′- IL-6, 5′- TCCAGTTGCCTTCTTGGGAC -3′; 3′- IL-6, 5′- GTGTAATTGCCTCCGACTTG -3′; 5′- GAPDH, 5′- TCCTGCACCACCAACT GCTTA -3′; 3′- GAPDH, 5' GTTCAGCTCTGGGATGACCTT −3′.

2.4. Enzyme-linked immunosorbent assay

Commercially available ELISA kits were used to detect the levels of tumor necrosis factor- α (TNF-α; #MTA00B; R&D Systems), Interluekin-6 (IL-6; #M6000B; R&D Systems) and Interleukin -1 β (IL-1β; #MLB00C; R&D Systems) in BV-2 microglial cells.BV-2 microglial cells and parkin-overexpressing BV-2 cells were cultured in 6-well plates (1×10^5cells/well), and then added LPS (1 μg/mL) for 6 and 12 hr. Then, the supernatants were collected after elimination of particulate matter by centrifugation at 3500 ×*g* for 2 min. Next, the cell culture supernatants were added into the corresponding wells, and the wells were sealed using adhesive tape and maintained at room temperature for 2 hr. A total of 100 μl biotinylated antibody fluids were supplemented into wells. Subsequently, the wells were sealed by adhesive tape and incubated for 2 hr at room temperature. Chromogenic substrate was added into the wells. Plates were incubated for 30 min in the dark at room temperature. Stop solution was added into each well and immediately mixed for 2 min at room temperature. The optical density (OD) at 450 nm was detected using a microplate reader (TECAN, Inc).

2.5. Western blot analysis

Whole protein lysates were obtained using PRO-PREP protein extraction solution (Intron Biotechnology, Korea). Cytoplasmic and mitochondrial fractions were isolated using a mitochondria isolation kit for cultured cells (Thermo Scientific, MA, USA) according to the manufacturer's protocol. Nuclear and cytoplasmic fractions were isolated using a NE-PER nuclear and cytoplasmic extraction reagent kit (Thermo Scientific) according to the manufacturer's protocol. Proteins were separated by electrophoresis on 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) systems and transferred onto nitrocellulose membranes (Pall Corporation, FL, USA). The transferred membranes were blocked with skimmed milk (5%) (Thermo Scientific). The membranes were then incubated overnight at 4 °C with either anti-parkin

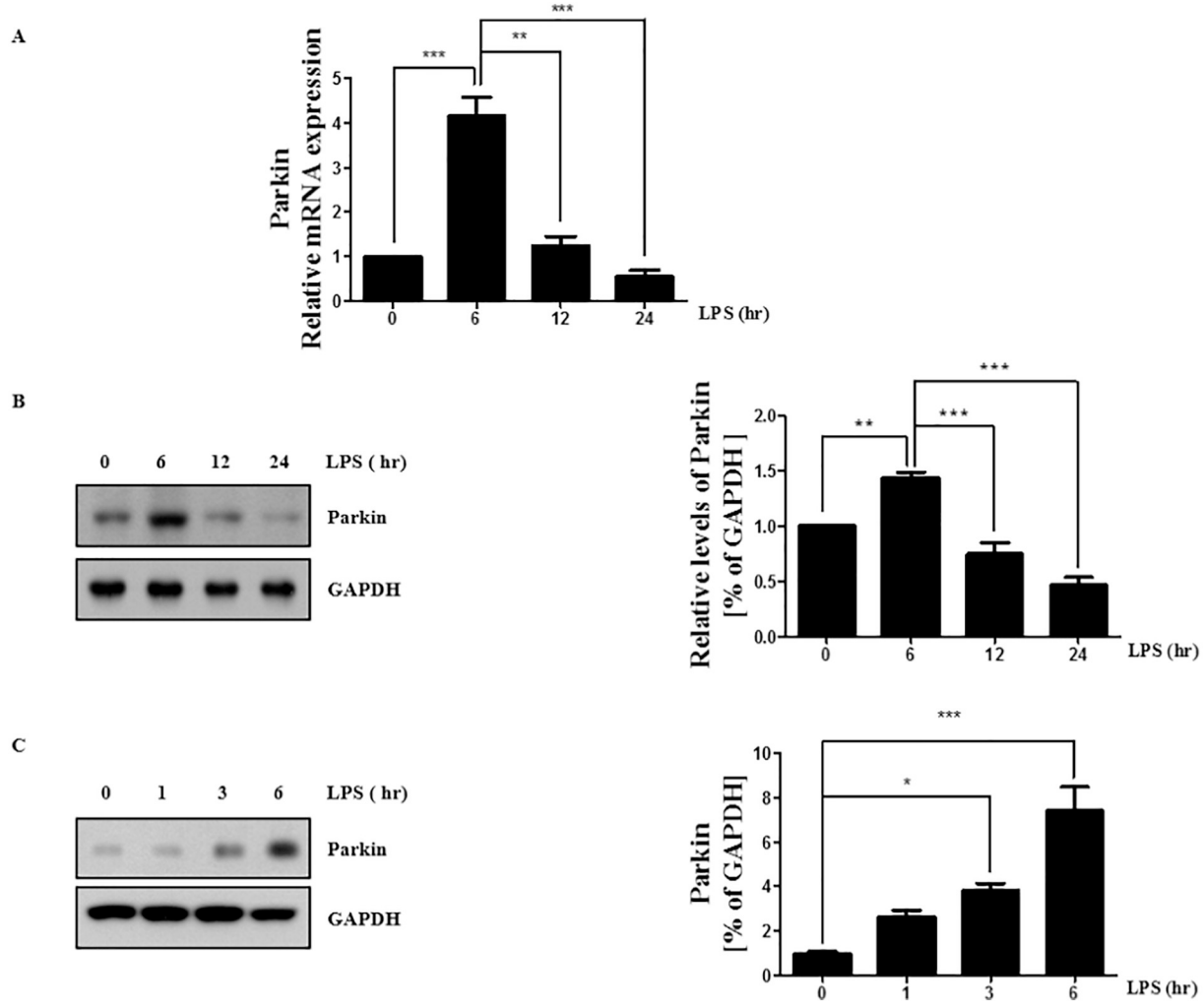

Fig. 1. Parkin is relevant to early-stage LPS-induced inflammatory responses.
(A) mRNA expression levels of parkin were observed in BV-2 microglial cells treated with LPS (1 μg/mL) for the indicated times (0, 6, 12, and 24 hr) through quantitative real-time PCR (qRT-PCR). (B) Proteins expression levels of parkin were observed in LPS treated BV-2 microglial cells for 0,6,12, and 24 hr through western blot analysis. (C) Protein expression levels of parkin were observed in BV-2 microglial cells following LPS stimulation for the indicated times (0, 1, 3, and 6 hr) through western blot analysis. (A), (B) and (C) used one-way ANOVA for statistical analysis. Data are presented as mean ± SD ($n = 3$). *$p < .05$, **$p < .01$, and ***$p < .001$.

(1:1000;#2132S), anti-iNOS (1:2000;#13120S), anti-COX-2 (1:2000; #1312A),anti-JNK (1:2000;#9252S), anti-phosphorylated (p)-JNK (1:1000;#9251S), anti-ERK (1:2000;9102S), anti-p-ERK (1:1000;9101S), anti-p38 (1:2000;9212S), anti-p-p38(1:800;4511S), anti-ubiquitin (1:1000;#3936S), anti-p62 (1:2000;#5114S), anti-LC3B (1:1000;#2775S), anti-COX-IV (1:1000;#4844S) (Cell Signaling, MA, USA), anti-PINK1 (1:1000; #23707) (Abcam, MA, USA), anti-GAPDH (1:4000;#1311F), anti-NF-κBp65 (11000;E0517), (Santa Cruz, TX, USA), or anti-Lamin B1 (14000;PA5-19468) (Invitrogen) primary antibodies. After washing with buffer, the membranes were incubated with horseradish peroxidase conjugated secondary antibodies (Thermo Scientific) at 4 ˚C overnight.

2.6. Nitric oxide detection

NO was measured in the supernatant by using a commercially available NO detection kit (Intron Biotechnology, Korea) according to the manufacturer's instructions. The absorbance of each tube was measured at 550 nm.

2.7. Immunocytochemistry

BV-2 cells ($\geq 1 \times 10^5$) were fixed with 4% paraformaldehyde (Sigma) for 1 hr and washed with cold phosphate-buffered saline (PBS). Then, the fixed cells were permeabilized with 0.25% Triton X-100 in PBS (0.25% PBST) for 10 min. After the permeabilization, fixed cells were washed with cold PBS and blocked with 0.25% PBST containing 1% bovine serum albumin. Then, the cells were incubated with anti-NF-κB (1:500; sc-8008, RRID: AB_628017; Santa Cruz), anti-IBA 1 (1:250; GT10312, MA5-27726; Invitrogen), and anti-LC3B antibodies (1:250; #2775S; Cell Signaling) at 4 ˚C overnight. The cells were then incubated with Alexa 488 goat anti-mouse secondary antibody, Alexa 350 goat anti-rabbit secondary antibody, and Alexa 488 goat anti-mouse secondary antibody (Thermo Scientific) at 4 ˚C overnight. Images were obtained using an LSM-710 confocal microscope (Carl Zeiss, Oberkochen, Germany).

2.8. Measurement of mitochondrial membrane potential

Mitochondrial- specific membrane potential was assessed using JC-1 dye (Thermo Scientific). BV-2 cells were harvested ($\geq 2 \times 10^5$) by using 0.05% Trypsin/EDTA with pre-warmed (37 ˚C) DMEM. Then, the cell suspension was centrifuged at 300 × g for 2 min. During the

centrifugation, an aliquot of 5 μM JC-1 stock solution was thawed. After that, the cell suspension was incubated with 5 μM JC-1 at 37 °C for 20 min in the dark, followed by washing with PBS and centrifugation at 300 ×*g* for 2 min. The cells were re-suspended in phenol red-free DMEM and analyzed with a FACS Verse flow cytometer (BD Biosciences).

2.9. Measurement of intracellular and mitochondrial ROS

Intracellular and mitochondrial ROS generation was assessed using CM-H_2DCF-DA and MitoSOX (Thermo Scientific). BV-2 cells were harvested ($\geq 2\times10^5$)using 0.05% Trypsin/EDTA with pre-warmed (37 °C) DMEM. Then, the cell suspension was centrifuged at 300 ×*g* for 2 min. During the centrifugation, an aliquot of 5 μM CM-H_2DCF-DA stock solution was thawed. Next, the cell suspension was incubated with 5 μM CM-H_2DCF-DA at 37 °C for 20 min in the dark, followed by washing PBS and centrifugation at 300 ×*g* for 2 min. The cells were re-suspended in phenol red-free DMEM and analyzed by flow cytometry (BD Biosciences).

2.10. . Statistical analysis

Prism software (GraphPad Prism version 5.0; La Jolla, CA, USA) was used for statistical analysis. Data represent mean ± SD of at least three independent experiments ($n \geq 3$). One-way analysis of variance (ANOVA) was used for group comparisons, followed by Dunnett's multiple comparison tests. A p-value < .05 was considered statistically significant and is indicated on graphs by an asterisk, whereas p- values < 0.01 and < 0.001 are indicated by two and three asterisks, respectively.

3. Results

3.1. Parkin is relevant to early-stage LPS-induced inflammatory responses

Before determining the interaction between Parkin and pro-inflammatory mediators, we observed the expression of parkin in LPS-induced microglial activation, which occurs via toll-like receptor 4

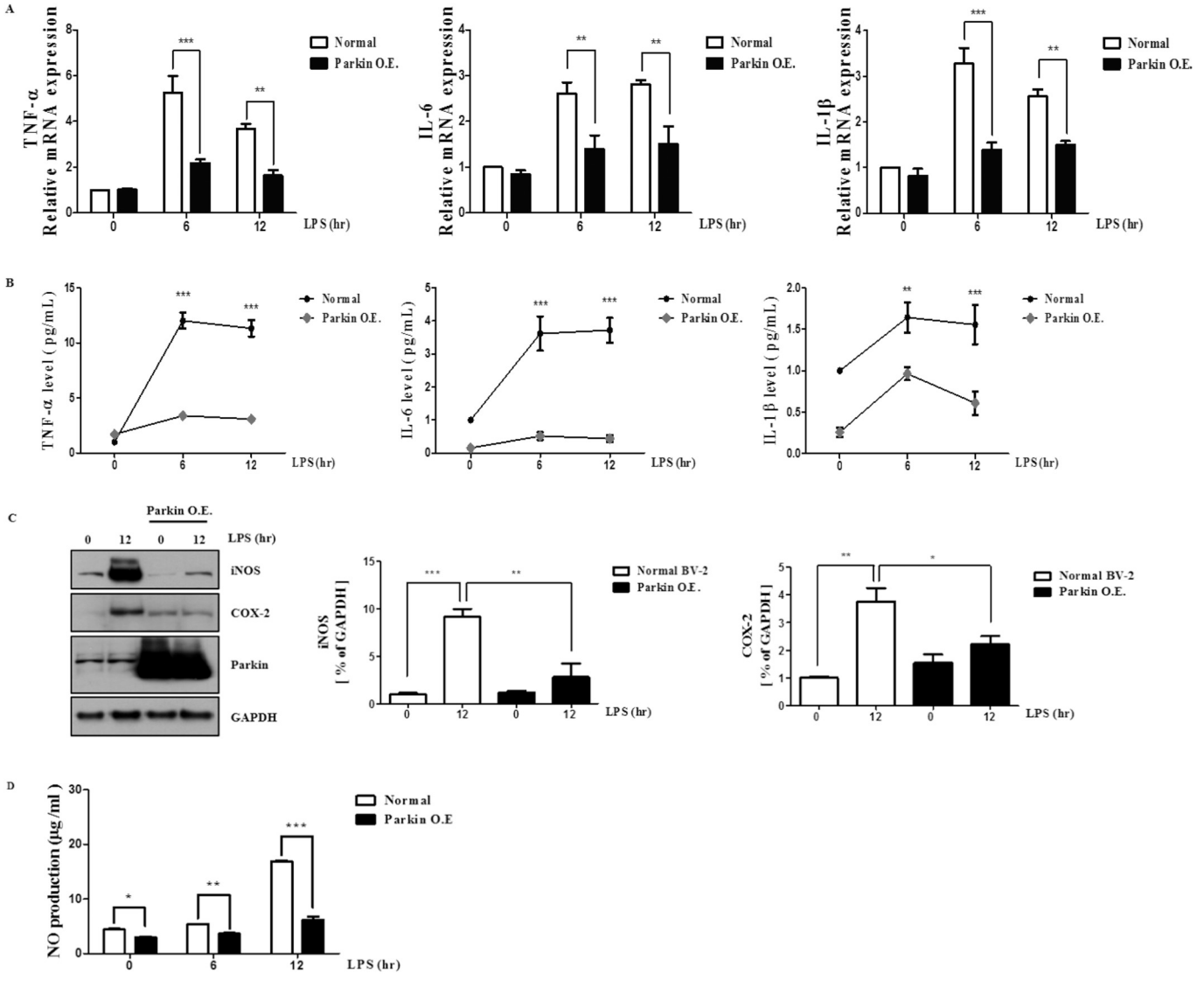

Fig. 2. Overexpressed parkin suppresses the production of pro-inflammatory cytokines and NO production in BV-2 microglial cells.
(A) mRNA levels of pro-inflammatory cytokines (TNF α, IL-1 β, and IL-6) were observed after stimulation with LPS (1 μg/mL) in BV-2 and parkin-overexpressing BV-2 microglial cells for the indicated times (0, 6, and 12 hr) through quantitative real-time PCR (qRT-PCR). (B) ELISA of pro-inflammatory cytokines (TNF α, IL-1 β, and IL-6) in LPS-stimulated BV-2 and parkin-overexpressing BV-2 microglial cells for the indicated times (0, 6, and 12 hr). (C) Western blot analysis of iNOS, COX-2, and Parkin in LPS-stimulated BV-2 and parkin-overexpressing BV-2 microglial cells for 12 hr. (D) Nitrite levels in control and parkin-overexpressing BV-2 microglial cells treated with LPS (1 μg/mL) for the indicated times (0, 6, and 12 hr). (B) Analyzed using one-way ANOVA and (A), (C) analyzed using two-way ANOVA for statistical analysis. Data are presented as mean ± SD ($n = 3$). *$p < .05$, **$p < .01$, and ***$p < .001$.

(TLR4) (Lehnardt, 2010), in BV-2 microglial cells, a model cell widely used in neuroinflammation research (Blasi et al., 1990).We first examined the mRNA levels of parkin in BV-2 microglial cells with LPS and found that it accrued until 6 hr and decreased gradually from 12 hr after the activation (Fig. 1A).We also investigated the protein levels of parkin (Fig. 1B). Western blot analysis showed a similar tendency for its mRNA levels. Further, we investigated the expression of parkin before 6 hr in the protein levels (Fig. 1C). As surmised, the protein levels of parkin augmented after LPS stimulation until 6 hr. Therefore, we concluded that the expression of parkin increased early on (until 6 hr) and decreased later (after 12 hr) in microglial activation. Overall, these results suggested that expression of parkin must be regulated at the transcriptional level.

3.2. Overexpressed parkin suppresses the production of pro-inflammatory cytokines and NO production in BV-2 microglial cells

To investigate the role of augmented parkin in the early stages of microglial activation and microglial pro-inflammatory responses, we built the lentivirus (pLenti6.3 Parkin-eGFP) and infected BV-2 cells to construct parkin-overexpressing stable cell line in BV-2 microglial cells. These cells and normal BV-2 microglial cells were stimulated with LPS.

In Fig. 1, parkin was elevated in the early stage of LPS-induced inflammatory responses. LPS also causes enhanced production of pro-inflammatory cytokines such as tumor necrosis factor- alpha (TNF-α), interleukin-1β (IL-1 β), interleukin-6 (IL-6), and NO (Fang et al., 2004). The mRNA levels of these cytokines were increased in the LPS-stimulated BV-2 microglial cells but decreased in parkin-overexpressing LPS-stimulated BV-2 microglial cells (Fig. 2A). Next, we confirmed lentivirus-mediated DsRed2-mito expressing BV-2 microglial cells through confocal fluorescence images (Supplementary Fig. S1) and excluded that some of the effects ascribed to parkin overexpression are in fact due to the prior viral infection by using lentivirus-mediated DsRed2-mito expressing (Supplementary Fig. S2). Additionally, we investigated the effect of overexpression of parkin against cytokines' expression in BV-2 microglial cells by using ELISA. Similarly, the production of TNF-α, IL-1 β, and IL-6 was hampered in parkin-overexpressing LPS-stimulated BV-2 microglial cells (Fig. 2B). We next examined the protein levels of inducible-NO (iNOS) and cyclooxygenase-2 (COX-2), which are well- known pro-inflammatory mediators and related with microglial activation (Petrova et al., 1999) (Fig. 2C). These were dramatically decreased in parkin-overexpressing LPS-stimulated BV-2 microglial cells and the effects of the prior viral infection were also excluded by lentivirus-mediated DsRed2-mito expressing (Supplementary Fig. S3).

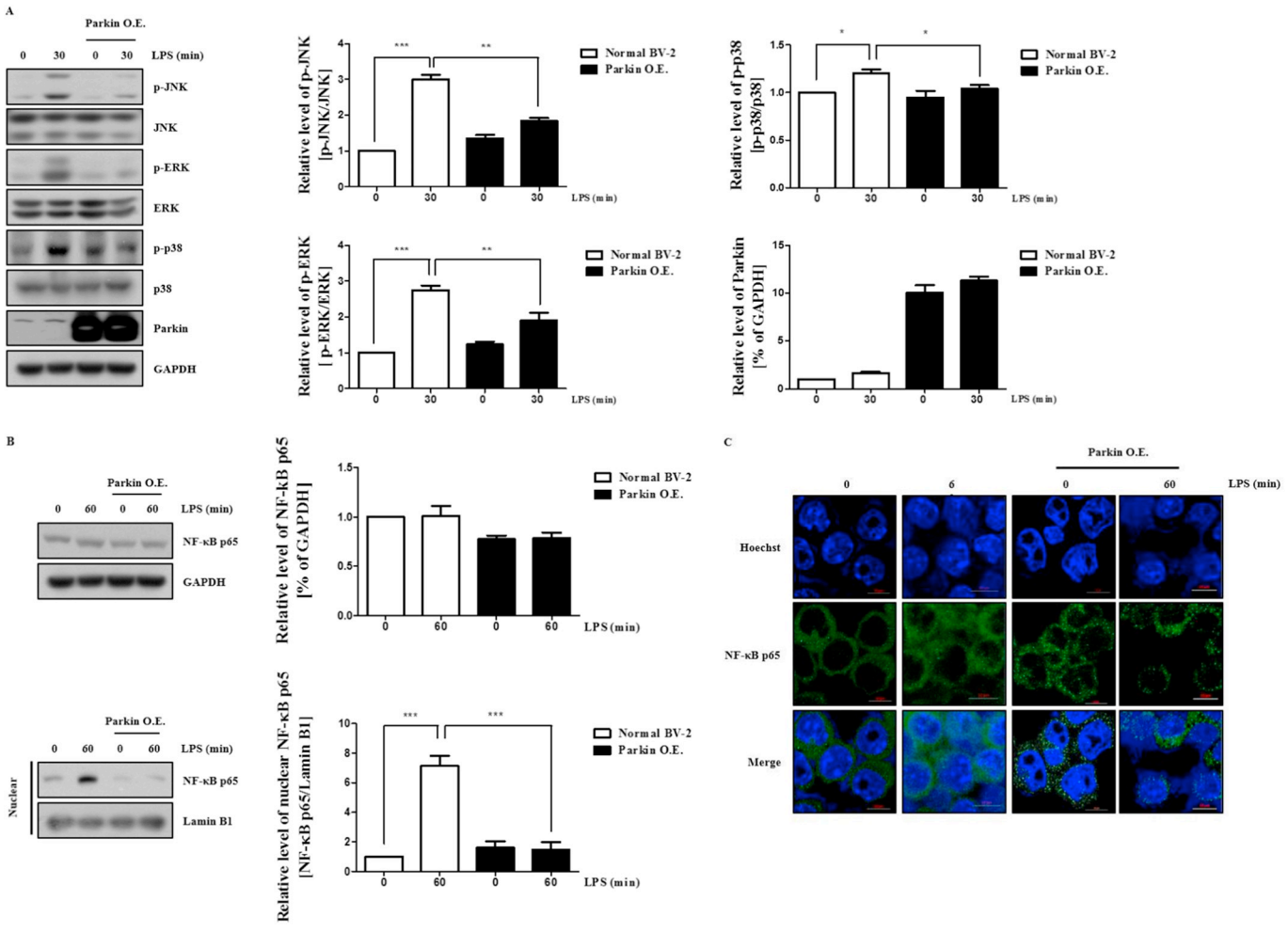

Fig. 3. Parkin controls LPS-induced pro-inflammatory responses in microglial cells via differential regulation of MAPK.
(A) Levels of (p)-JNK, (p)-ERK, and (p)-p38 in the whole cell lysates were evaluated by western blotting in BV-2 - and BV-2 parkin-overexpressing microglial cells after LPS stimulation (1 μg/mL) for the indicated times (0 and 30 min). (B) Levels of NF-κB p65 in the whole cell and nuclear fraction were evaluated by western blotting in BV-2 - and BV-2 parkin-overexpressing microglial cells after LPS stimulation (1 μg/mL) for the indicated times (0 and 60 min). (C) BV-2- and parkin-overexpressing cells were stimulated with LPS. The translocation level of NF-κB p65 was determined by immunocytochemistry. Nuclei are stained with Hoechst, and the merged images show the NF-κB and Hoechst signals. Scale bar, 10 μm. (A), (B) used one-way ANOVA for statistical analysis. Data are presented as mean ± SD ($n = 3$). *$p < .05$, **$p < .01$, and ***$p < .001$.

Finally, we investigated whether parkin suppresses the production of NO in LPS-stimulated BV-2 microglial cells (Fig. 2D). Parkin-overexpressing BV-2 microglial cells showed substantially decreased levels of NO production compared with normal BV-2 microglial cells. These results suggest that increased levels of parkin in the early stage alleviated microglial pro-inflammatory responses.

3.3. Parkin controls LPS-induced pro-inflammatory responses in microglial cells via differential regulation of MAPK

Numerous studies have shown that activation of MAPKs, such as JNK, ERK, and p38 play an important role in modulating the expression of pro-inflammatory cytokines/molecules in stimulated microglia (Kim et al., 2004; Park et al., 2015; Torres, 2003). We examined the effects of parkin overexpression on the activity of JNK, ERK1/2, and p38 in LPS-activated BV-2 microglial cells. Our results showed that LPS activation of JNK, ERK, and p38 was down-regulated by parkin overexpression. JNK and ERK activation was markedly decreased by Parkin overexpression. (Fig. 3A); p38 not as much. In addition to MAPKs, the existing literature considered the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway as a pertinent factor in the expression of pro-inflammatory genes including cytokines/chemokines and inflammasome regulation (Lawrence, 2009; Ye et al., 2017). To test the effect of overexpressed parkin on NF-κB p65, we observed the protein levels of NF-κB p65 in the whole cell lysates. The expression of NF-κB p65 did not show any differences between control and parkin-overexpressing cells. Accordingly, we isolated the cytoplasm and nuclear fraction from BV-2 microglial cells and investigated the protein levels of NF-κB p65 in the nuclear fraction. The increased LPS-stimulated NF-κB p65 in BV-2 microglial cells was considerably reduced in parkin-overexpressing BV-2 microglial cells (Fig. 3B). Furthermore, we confirmed the subcellular localization of NF-κB p65 in control- and parkin-overexpressing BV-2 cells after LPS stimulation by confocal microscopy. Our data showed that the translocation of NF-κB p65 into the nuclei was notably decreased in parkin-overexpressing cells compared to controls (Fig. 3C). Taken together, overexpression of parkin regulates the pro-inflammatory response in LPS-stimulated microglial cells by modulating MAPK, especially the JNK, ERK, and NF-κB p65 signal pathways.

3.4. Parkin is involved in the early stage of mitophagy in activated BV-2 microglial cells

Our previous data showed that parkin modulated the production and secretion of pro-inflammatory cytokines and NO to maintain cellular quality via down-regulation of MAPK and NF-κB signals. Accordingly, we hypothesized that parkin must be relevant to mitophagy, a well-known mechanism for mitochondrial clearance, in activated BV-2 microglial cells. We detected PINK1, Parkin, ubiquitin, p62, and LC3B, major components of the mitophagic process, in the mitochondrial fraction of LPS-stimulated BV-2 microglial cells (Fig. 4A). Our data showed that the protein levels of PINK1 and Parkin were much more increased in early stages in the mitochondrial fraction. The ubiquitination patterns in the mitochondrial fraction were significantly increased as well. Furthermore, p62, a selective cargo receptor for autophagy and important for the regulation of mitophagy (Narendra et al., 2010; Song et al., 2016), and LC3B, which interacts with p62 to remove protein aggregates, was increased in the mitochondrial fraction. We next investigated whether overexpression of parkin can induce mitophagy following long-term LPS stimulation (24 hr) which parkin mostly decreased, and mitophagy therefore may not occur (Fig. 4B). Our data showed that decreased parkin and PINK 1 were significantly augmented, and LC3-II were increased slightly in parkin overexpression. In addition, the level of ubiquitination was considerably augmented in parkin overexpression, and p62 increased as well. Taken together, overexpression of parkin can induce delayed mitophagy when parkin must be decreased and mitophagy, therefore, may not be occurred. Next, we confirmed the subcellular localization of LC3B in BV-2 and parkin overexpressing cells after LPS stimulation by confocal microscopy (Fig. 4C). Our image data showed that the microglial activation marker Iba-1 was increased in a time-dependent manner with LPS, and the subcellular localization of LC3B into mitochondria was highest after 6 hr stimulation. Besides, the subcellular level of LC3B into the mitochondria was more increased in parkin-overexpressing cells. Based on these results, it seems likely that parkin-mediated mitophagy can occur early after LPS stimulation, and this event can be rescued even in late time by overexpression of parkin.

3.5. Parkin contributes to mitochondrial quality control through downregulation of ROS

Mitochondrial quality control (MQC) is pertinent to homeostasis in CNS. When damaged mitochondria accumulate, there is a drop in the mitochondrial membrane potential and excessive production of ROS (Suski et al., 2012). Thus, MQC cannot be maintained, and many organelles are damaged. We therefore investigated whether the overexpression of parkin can regulate mitochondrial membrane potential and intracellular/mitochondrial ROS, respectively. We used JC-1, a well-known mitochondrial membrane potential marker (Fig. 5A). The results showed that mitochondrial membrane potential of LPS-stimulated parkin-overexpressing BV-2 microglial cells was significantly salvaged. Next, we measured intracellular ROS by staining BV-2 microglial controls and parkin-overexpressing BV-2 cells with the indicator CM-H_2DCF-DA (Fig. 5B). Overexpression of parkin effectively suppressed LPS-upregulated ROS. We also measured mitochondrial-specific ROS by MitoSOX staining, which can be selectively oxidized by mitochondrial superoxide anions, in BV-2 microglial control and parkin-overexpressing BV-2 cells (Fig. 5C). Enhanced parkin considerably suppressed the elevated LPS-induced ROS. In short, LPS activated BV-2 microglial cells by causing mitochondrial ROS production and reducing mitochondrial membrane potential and eventually provoking, mitochondrial damage. Taken together, it is reasonable to conclude that parkin-mediated mitophagy functions as a scavenger of LPS-induced mitochondrial ROS through elimination of damaged mitochondria and restores disrupted mitochondrial quality, thereby suppressing the secretion of pro-inflammatory cytokines in BV-2 microglial cells.

4. Discussion

Several lines of evidence have demonstrated that loss of parkin sensitized cells, especially their mitochondria, to inflammatory responses. (Frank-Cannon et al., 2008; Hwang et al., 2017; Mouton-Liger et al., 2018). However, the relationship between parkin and LPS-induced inflammatory responses in microglial cells is not yet fully understood. In this study, we first showed that the expression of parkin is increased early and decreased late, at both the transcriptional and translational levels after LPS stimulation, a well-known inducer of microglial activation through TLR4. Parkin may have a critical role in alleviating inflammatory responses to maintain normal cellular conditions.

Inflammatory mediators released by the microglia have causative and exacerbating roles in neurodegenerative diseases. Many previous studies showed that inflammatory responses were increased when parkin was deficient (Frank-Cannon et al., 2008; Mouton-Liger et al., 2018). Thus, it is conceivable that parkin regulates pro-inflammatory responses. Our results showed that overexpression of parkin substantially suppressed translocation of NF-κB p65 to the nucleus and the production of pro-inflammatory cytokines and NO in BV-2 microglial cells in response to LPS. In line with these findings, parkin was suggested to regulate pro-inflammatory mediators in microglial cells. In this study, we showed that transcriptional levels of parkin are relevant

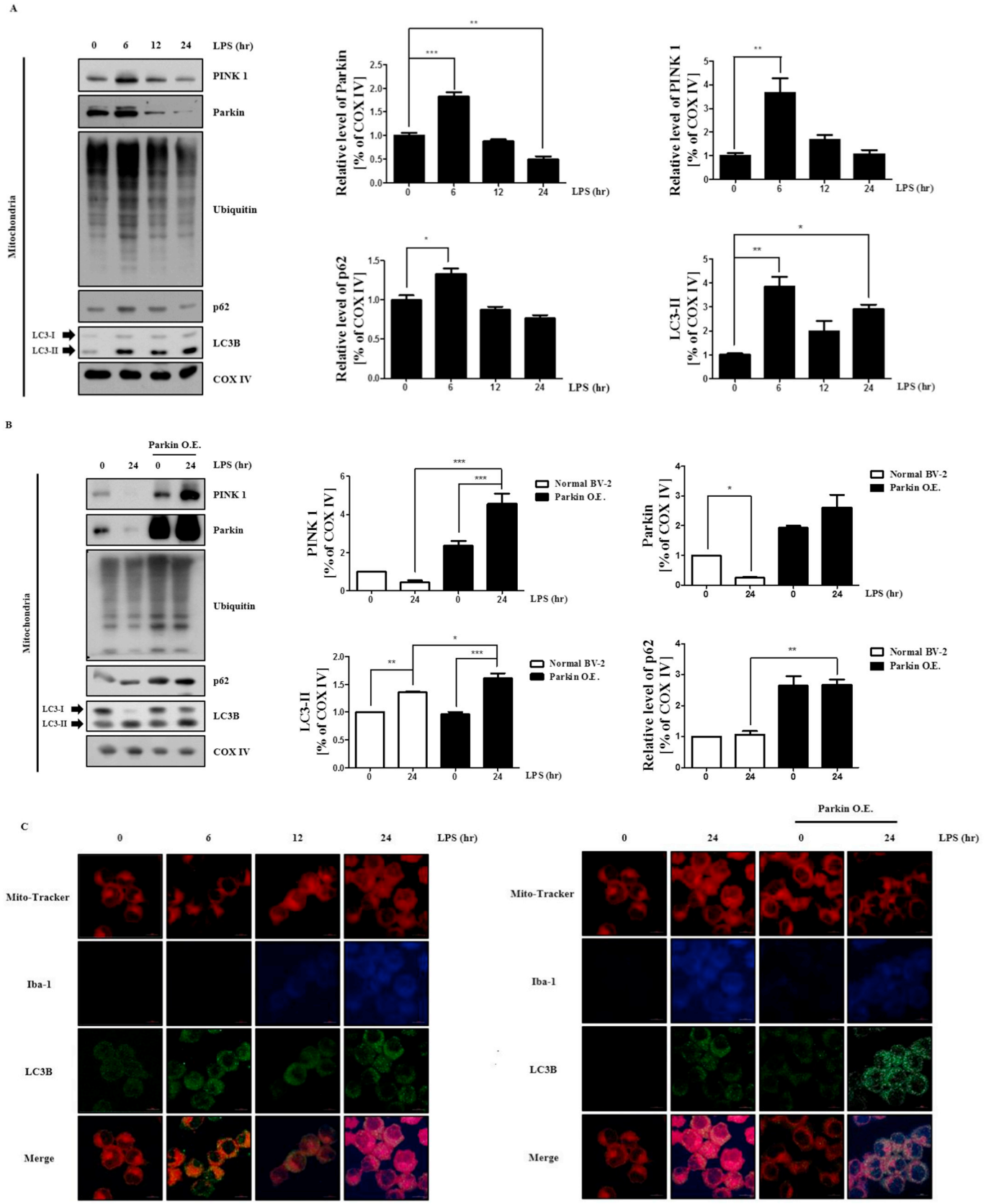

Fig. 4. Parkin is involved in the early stage of mitophagy in activated BV-2 microglial cells.
(A) Levels of PINK1, Parkin, Ubiquitin, p62, and LC3B in the mitochondrial fraction were evaluated by western blotting in BV-2 microglial cells stimulated for the indicated times (0, 6, 12, 24 hr) with LPS (1 μg/mL). (B) PINK 1, Ubiquitin, p62, and LC3B levels were examined by western blotting after stimulation of LPS for 24 hr in BV-2 and parkin-overexpressing cells. (C) LC3B levels in the mitochondria were determined by immunocytochemistry. Mitochondria were stained with Mito-Tracker Red, and Iba-1 was used as the microglial activation marker. The merged images show the Iba-1, LC3B, and Mito-Tracker signals. COX IV was used as mitochondrial loading controls. Data were analyzed using two-way ANOVA. Data are presented as mean ± SD ($n = 3$). *$p < .05$, **$p < .01$, and ***$p < .001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

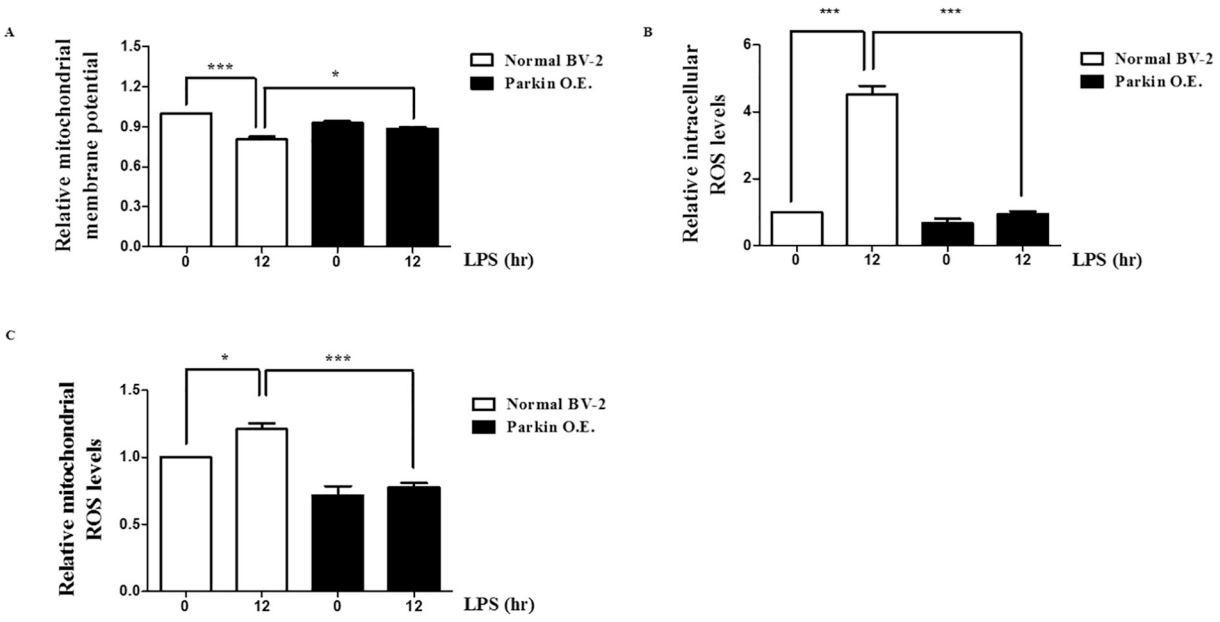

Fig. 5. Parkin contributes to mitochondrial quality control through downregulation of reactive oxygen species.
Controland parkin-overexpressing BV-2 microglial cells were treated with LPS (1 μg/mL) for 12 hr. The cells were incubated with JC-1, CM-H_2DCF-DA, and MitoSOX for 15 min at 37 °C, and mitochondrial membrane potential indicator (JC-1) (A), intracellular ROS (B), and mitochondrial ROS (C) were analyzed by flow cytometry. (A), (B), and (C) used one-way ANOVA for statistical analysis. Data are presented as mean ± SD ($n = 3$). *$p < .05$, **$p < .01$, and ***$p < .001$.

to early-stage LPS-induced inflammatory responses. Although the precise mechanism of transcriptional regulation is not yet elucidated, Tran et al. suggested that LPS-induced NF-κB binds to the *parkin* promoter to repress transcriptional activity, inducing excessive inflammatory response (Tran et al., 2011). However, our data indicated that LPS-induced oxidative stresses in the early stage led to parkin expression that must be related to inflammatory pathways. Letsiou et al. reported that parkin regulates LPS-induced pro-inflammatory response in acute lung injury (Letsiou et al., 2017). Our results suggested that parkin might be involved in early pro-inflammatory responses, resulting in regulation of microglial cell activation. Therefore, regulation of parkin is important to preserve the basal level of inflammatory responses. Winklhofer and his research group suggested that parkin initiates a neuroprotective program under moderate stress by regulating the NF-kB pathway in human neuroblastoma SH-SY5Y cells (Henn et al., 2007). Besides, they identified parkin as an additional component of linear ubiquitin chain assembly complex (LUBAC), which induces linear ubiquitination of NF-κB essential modulator (NEMO) to prevent stress-induced cell death (Muller-Rischart et al., 2013). However, our results showed that overexpression of parkin suppress the translocation of NF-kB after LPS stimulation; thus, it depends on the cell type, stimuli, duration of the stimulus, and other cellular conditions. The precise mechanism between parkin and MAPKs has not yet fully understood. Therefore, our future study plans to focus on the mechanism of interaction between parkin and MAPK by investigating how parkin can be regulated at the levels of transcription/translation in microglial cells after LPS stimulation.

Mounting evidence has demonstrated that parkin-mediated mitophagy is important to remove damaged mitochondria and maintain cellular homeostasis (Eiyama and Okamoto, 2015; Vigie and Camougrand, 2017; Youle and Narendra, 2011). Mitophagy degrades damaged mitochondria and helps to maintain the MQC (Pickles et al., 2018; Vigie and Camougrand, 2017). One of the most studied mechanisms for mitophagy in mammalian cells is the parkin-mediated mitophagy pathway (Eiyama and Okamoto, 2015; Youle and Narendra, 2011). Parkin is a cytosolic E3 ubiquitin ligase that is involved in a common pathway regulating MQC and inducing mitophagy (Narendra et al., 2012; Youle and Narendra, 2011). Most of the existing literature pertaining to the role of parkin and mitophagy is focused on neurons (Ashrafi et al., 2014; Ebrahimi-Fakhari et al., 2016), and little is known about its role in microglial cells, which are responsible for most inflammatory responses in the CNS. In LPS-stimulated microglial cells, our results showed that the expressions of PINK1, Parkin, ubiquitin, p62, and LC3B were more increased in the early stage in the mitochondrial fraction. In other words, the expression of parkin in early LPS-stimulated BV-2 microglial cells may induce mitophagy to regulate pro-inflammatory responses. We also confirmed that delayed mitophagy can occur, which Parkin mostly decreased therefore mitophagy may not be occurred, via overexpression of Parkin.

Recently, Hwang et al. showed that parkin markedly suppresses both *Hep* (Drosophila JNK kinase) and *Bsk* (Drosophila JNK) via transcriptional regulation (Hwang et al., 2010). In addition, Hirota et al. showed that induction of mitophagy relies on MAPK1/ERK2 and MAPK14/p38 (Hwang, Kim et al., 2010). However, little is known about the interaction between parkin and MAPKs in microglial cells. In this study, we showed that overexpressed parkin regulates MAPKs activation induced by LPS stimulation. Interestingly, parkin markedly down-regulates the activation of JNK and ERK compared with p38 in microglial cells. Recent studies have showed that p38 MAPK negatively regulates parkin by phosphorylating parkin in serine 131 and suppresses the interaction between parkin and PINK1, thereby causing decreased mitophagy (Chen et al., 2018). Thus, it is conceivable that p38 may be involved in the upstream regulation of parkin. Besides, to assess the effect of parkin on MAPKs, we pre-treated cells with U0126 (10 μM), SP600125 (20 μM), and SB203580 (10 μM) to inhibit ERK, JNK, and p38, respectively. Intriguingly, ERK showed increased more activation when cells were pre-treated with SB203580 and SP600125; parkin expression showed a decrease upon treatment with U0126, SP600125, and SB203580 (data not shown). Parkin in microglial cells mainly appears to regulate the activation of JNK and ERK rather than p38. However, the mechanism between parkin and MAPK still remains elusive, and future studies are needed to elucidate the precise mechanisms of interaction between parkin and other MAPKs.

Our previous study demonstrated that mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglial cells by regulating the MAPK and NF-κB pathways (Park et al., 2015). LPS-induced

immoderate ROS aggravate cellular homeostasis, especially by inducing the defective turnover of mitochondria, which sequentially provoked the accumulation of damaged mitochondria. In turn, the plethora of damaged mitochondria produce excessive mitochondrial ROS (mtROS) and cause the loss of mitochondrial membrane potential, events that are directly related to the mitochondrial dysfunction that could, in turn, induce mitophagy (Fischer et al., 2012; Youle and Narendra, 2011). Therefore, proper regulation of ROS by mitophagy is crucial for cell viability. In this study, we showed that overexpression of parkin mitigates mitochondrial membrane potential, intracellular ROS, and mtROS, and thus ameliorates damaged mitochondria in microglial cells. Recent studies have shown that inhibition of mitophagy induces NLRP3 inflammasome activation by increasing superoxide production (Zhou et al., 2011); the NLRP3 inflammasome is involved in neuroinflammation (Freeman and Ting, 2016). It is becoming increasingly evident that mtROS in microglia is highly associated with the NLRP3 inflammasome, though the link between mitophagy and neuroinflammation in microglia still needs to be clarified.

From the results of this study, we speculate that accumulation of damaged mitochondria plays a causative and exacerbating role in disruption of microglial homeostasis, and that parkin regulates mitochondrial homeostasis via the production of early pro-inflammatory mediators by circumventing LPS-induced MAPK and NF-κB activation in microglial cells. Our results indicate that regulation of parkin expression helps prevent neuroinflammation caused by activated microglial cells. Further studies should be carried out to better understand the interplay between mitophagy and microglial inflammatory responses both under physiological and pathological conditions for approaches to prevent neurodegenerative diseases in which microglial cells play a pertinent role.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.577044.

References

Ashrafi, G., Schlehe, J.S., LaVoie, M.J., Schwarz, T.L., 2014. Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. J. Cell Biol. 206, 655–670.
Bamford, S., Ryley, H., Jackson, S.K., 2007. Highly purified lipopolysaccharides from Burkholderia cepacia complex clinical isolates induce inflammatory cytokine responses via TLR4-mediated MAPK signalling pathways and activation of NFkappaB. Cell. Microbiol. 9, 532–543.
Banati, R.B., Egensperger, R., Maassen, A., Hager, G., Kreutzberg, G.W., Graeber, M.B., 2004. Mitochondria in activated microglia in vitro. J. Neurocytol. 33, 535–541.
Blasi, E., Barluzzi, R., Bocchini, V., Mazzolla, R., Bistoni, F., 1990. Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus. J. Neuroimmunol. 27, 229–237.
Block, M.L., Hong, J.S., 2007. Chronic microglial activation and progressive dopaminergic neurotoxicity. Biochem. Soc. Trans. 35, 1127–1132.
Cargnello, M., Roux, P.P., 2011. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol. Mol. Biol. Rev. 75, 50–83.
Casano, A.M., Peri, F., 2015. Microglia: multitasking specialists of the brain. Dev. Cell 32, 469–477.
Chen, J., Ren, Y., Gui, C., Zhao, M., Wu, X., Mao, K., et al., 2018. Phosphorylation of Parkin at serine 131 by p38 MAPK promotes mitochondrial dysfunction and neuronal death in mutant A53T alpha-synuclein model of Parkinson's disease. Cell Death Dis. 9, 700.
Cornelissen, T., Vilain, S., Vints, K., Gounko, N., Verstreken, P., Vandenberghe, W., 2018. Deficiency of parkin and PINK1 impairs age-dependent mitophagy in Drosophila. Elife. 7.
Czarna, M., Jarmuszkiewicz, W., 2006. Role of mitochondria in reactive oxygen species generation and removal; relevance to signaling and programmed cell death. Postepy Biochem. 52, 145–156.
Dello Russo, C., Boullerne, A.I., Gavrilyuk, V., Feinstein, D.L., 2004. Inhibition of microglial inflammatory responses by norepinephrine: effects on nitric oxide and interleukin-1beta production. J. Neuroinflammation 1, 9.
Durcan, T.M., Fon, E.A., 2015. The three 'P's of mitophagy: PARKIN, PINK1, and post-translational modifications. Genes Dev. 29, 989–999.
Ebrahimi-Fakhari, D., Saffari, A., Wahlster, L., Di Nardo, A., Turner, D., Lewis Jr., T.L., et al., 2016. Impaired mitochondrial dynamics and Mitophagy in neuronal models of tuberous sclerosis complex. Cell Rep. 17, 1053–1070.
Eiyama, A., Okamoto, K., 2015. PINK1/Parkin-mediated mitophagy in mammalian cells. Curr. Opin. Cell Biol. 33, 95–101.
Fang, H., Pengal, R.A., Cao, X., Ganesan, L.P., Wewers, M.D., Marsh, C.B., et al., 2004. Lipopolysaccharide-induced macrophage inflammatory response is regulated by SHIP. J. Immunol. 173, 360–366.
Fischer, F., Hamann, A., Osiewacz, H.D., 2012. Mitochondrial quality control: an integrated network of pathways. Trends Biochem. Sci. 37, 284–292.
Frank-Cannon, T.C., Tran, T., Ruhn, K.A., Martinez, T.N., Hong, J., Marvin, M., et al., 2008. Parkin deficiency increases vulnerability to inflammation-related nigral degeneration. J. Neurosci. 28, 10825–10834.
Freeman, L.C., Ting, J.P., 2016. The pathogenic role of the inflammasome in neurodegenerative diseases. J. Neurochem. 136, 29–38 Suppl 1.
Gao, H.M., Zhou, H., Hong, J.S., 2012. NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. Trends Pharmacol. Sci. 33, 295–303.
Harper, A.E., Miller, R.H., Block, K.P., 1984. Branched-chain amino acid metabolism. Annu. Rev. Nutr. 4, 409–454.
Henn, I.H., Bouman, L., Schlehe, J.S., Schlierf, A., Schramm, J.E., Wegener, E., et al., 2007. Parkin mediates neuroprotection through activation of IkappaB kinase/nuclear factor-kappaB signaling. J. Neurosci. 27, 1868–1878.
Hwang, S., Kim, D., Choi, G., An, S.W., Hong, Y.K., Suh, Y.S., et al., 2010. Parkin suppresses c-Jun N-terminal kinase-induced cell death via transcriptional regulation in Drosophila. Mol. Cell 29, 575–580.
Hwang, C.J., Kim, Y.E., Son, D.J., Park, M.H., Choi, D.Y., Park, P.H., et al., 2017. Parkin deficiency exacerbate ethanol-induced dopaminergic neurodegeneration by P38 pathway dependent inhibition of autophagy and mitochondrial function. Redox Biol. 11, 456–468.
Kaminska, B., 2005. MAPK signalling pathways as molecular targets for anti-inflammatory therapy–from molecular mechanisms to therapeutic benefits. Biochim. Biophys. Acta 1754, 253–262.
Kastaniotis, A.J., Autio, K.J., Keratar, J.M., Monteuuis, G., Makela, A.M., Nair, R.R., et al., 2017. Mitochondrial fatty acid synthesis, fatty acids and mitochondrial physiology. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1862, 39–48.
Kim, E.K., Choi, E.J., 2010. Pathological roles of MAPK signaling pathways in human diseases. Biochim. Biophys. Acta 1802, 396–405.
Kim, S.H., Smith, C.J., Van Eldik, L.J., 2004. Importance of MAPK pathways for microglial pro-inflammatory cytokine IL-1 beta production. Neurobiol. Aging 25, 431–439.
Kim, T.S., Choi, H.S., Ryu, B.Y., Gang, G.T., Kim, S.U., Koo, D.B., et al., 2010. Real-time in vivo bioluminescence imaging of lentiviral vector-mediated gene transfer in mouse testis. Theriogenology 73, 129–138.
Komander, D., Rape, M., 2012. The ubiquitin code. Annu. Rev. Biochem. 81, 203–229.
Lawrence, T., 2009. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb. Perspect. Biol. 1, a001651.
Lehnardt, S., 2010. Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neuronal injury. Glia. 58, 253–263.
Letsiou, E., Sammani, S., Wang, H., Belvitch, P., Dudek, S.M., 2017. Parkin regulates lipopolysaccharide-induced proinflammatory responses in acute lung injury. Transl. Res. 181, 71–82.
Lull, M.E., Block, M.L., 2010. Microglial activation and chronic neurodegeneration. Neurotherapeutics 7, 354–365.
Mortiboys, H., Thomas, K.J., Koopman, W.J., Klaffke, S., Abou-Sleiman, P., Olpin, S., et al., 2008. Mitochondrial function and morphology are impaired in parkin-mutant fibroblasts. Ann. Neurol. 64, 555–565.
Mouton-Liger, F., Rosazza, T., Sepulveda-Diaz, J., Ieang, A., Hassoun, S.M., Claire, E., et al., 2018. Parkin deficiency modulates NLRP3 inflammasome activation by attenuating an A20-dependent negative feedback loop. Glia. 66 (8), 1736–1751.
Muftuoglu, M., Elibol, B., Dalmizrak, O., Ercan, A., Kulaksiz, G., Ogus, H., et al., 2004. Mitochondrial complex I and IV activities in leukocytes from patients with parkin mutations. Mov. Disord. 19, 544–548.
Muller-Rischart, A.K., Pilsl, A., Beaudette, P., Patra, M., Hadian, K., Funke, M., et al., 2013. The E3 ligase parkin maintains mitochondrial integrity by increasing linear ubiquitination of NEMO. Mol. Cell 49, 908–921.
Murphy, M.P., 2009. How mitochondria produce reactive oxygen species. Biochem. J.

417, 1–13.
Narendra, D., Kane, L.A., Hauser, D.N., Fearnley, I.M., Youle, R.J., 2010. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. Autophagy. 6, 1090–1106.
Narendra, D., Walker, J.E., Youle, R., 2012. Mitochondrial quality control mediated by PINK1 and Parkin: links to parkinsonism. Cold Spring Harb. Perspect. Biol. 4.
Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 308, 1314–1318.
Olajide, O.A., Aderogba, M.A., Fiebich, B.L., 2013. Mechanisms of anti-inflammatory property of Anacardium occidentale stem bark: inhibition of NF-kappaB and MAPK signalling in the microglia. J. Ethnopharmacol. 145, 42–49.
Palacino, J.J., Sagi, D., Goldberg, M.S., Krauss, S., Motz, C., Wacker, M., et al., 2004. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J. Biol. Chem. 279, 18614–18622.
Park, J., Min, J.S., Kim, B., Chae, U.B., Yun, J.W., Choi, M.S., et al., 2015. Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-kappaB pathways. Neurosci. Lett. 584, 191–196.
Park, H.J., Min, S.H., Choi, H., Park, J., Kim, S.U., Lee, S., et al., 2016. Mitochondria-targeted DsRed2 protein expression during the early stage of bovine somatic cell nuclear transfer embryo development. In Vitro Cell Dev. Biol. Anim. 52, 812–822.
Petrova, T.V., Akama, K.T., Van Eldik, L.J., 1999. Selective modulation of BV-2 microglial activation by prostaglandin E(2). Differential effects on endotoxin-stimulated cytokine induction. J. Biol. Chem. 274, 28823–28827.
Pickles, S., Vigie, P., Youle, R.J., 2018. Mitophagy and quality control mechanisms in mitochondrial maintenance. Curr. Biol. 28 (R170-R85).
Prinz, M., Priller, J., 2014. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nat. Rev. Neurosci. 15, 300–312.
Ransohoff, R.M., Brown, M.A., 2012. Innate immunity in the central nervous system. J. Clin. Invest. 122, 1164–1171.
Rojo, A.I., McBean, G., Cindric, M., Egea, J., Lopez, M.G., Rada, P., et al., 2014. Redox control of microglial function: molecular mechanisms and functional significance. Antioxid. Redox Signal. 21, 1766–1801.
Seirafi, M., Kozlov, G., Gehring, K., 2015. Parkin structure and function. FEBS J. 282, 2076–2088.
Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., et al., 2000. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat. Genet. 25, 302–305.
Song, P., Li, S., Wu, H., Gao, R., Rao, G., Wang, D., et al., 2016. Parkin promotes proteasomal degradation of p62: implication of selective vulnerability of neuronal cells in the pathogenesis of Parkinson's disease. Protein Cell. 7, 114–129.
Stehling, O., Wilbrecht, C., Lill, R., 2014. Mitochondrial iron-sulfur protein biogenesis and human disease. Biochimie 100, 61–77.
Suski, J.M., Lebiedzinska, M., Bonora, M., Pinton, P., Duszynski, J., Wieckowski, M.R., 2012. Relation between mitochondrial membrane potential and ROS formation. Methods Mol. Biol. 810, 183–205.
Torres, M., 2003. Mitogen-activated protein kinase pathways in redox signaling. Front. Biosci. 8, d369–d391.
Tran, T.A., Nguyen, A.D., Chang, J., Goldberg, M.S., Lee, J.K., Tansey, M.G., 2011. Lipopolysaccharide and tumor necrosis factor regulate Parkin expression via nuclear factor-kappa B. PLoS One 6, e23660.
Vigie, P., Camougrand, N., 2017. Role of mitophagy in the mitochondrial quality control. Med. Sci. (Paris) 33, 231–237.
von Bernhardi, R., Eugenin-von Bernhardi, L., Eugenin, J., 2015. Microglial cell dysregulation in brain aging and neurodegeneration. Front. Aging Neurosci. 7, 124.
West, A.P., Shadel, G.S., Ghosh, S., 2011. Mitochondria in innate immune responses. Nat. Rev. Immunol. 11, 389–402.
Ye, J., Jiang, Z., Chen, X., Liu, M., Li, J., Liu, N., 2017. The role of autophagy in pro-inflammatory responses of microglia activation via mitochondrial reactive oxygen species in vitro. J. Neurochem. 142, 215–230.
Youle, R.J., Narendra, D.P., 2011. Mechanisms of mitophagy. Nat. Rev. Mol. Cell Biol. 12, 9–14.
Zavodnik, I.B., 2016. Mitochondria, calcium homeostasis and calcium signaling. Biomed. Khim. 62, 311–317.
Zhou, R., Yazdi, A.S., Menu, P., Tschopp, J., 2011. A role for mitochondria in NLRP3 inflammasome activation. Nature. 469, 221–225.