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Original Article

PARK2 attenuates house dust mite-induced inflammatory reaction, pyroptosis and barrier dysfunction in BEAS-2B cells by ubiquitinating NLRP3

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Abstract: Objective: PARK2, a Parkinson's disease-associated gene, functions as an E3 ubiquitin ligase regulating the degradation of proteins via ubiquitination. Our study was designed to explore its role in allergic asthma and the underlying mechanisms. Methods: Airway epithelial cell line BEAS-2B was treated with house dust mite (HDM) to mimic allergic asthma in vitro. Lentivirus oePARK2 and siPARK2 were constructed to overexpress and knock down PARK2 expression, respectively. RT-qPCR, western blot, co-immunoprecipitation, and ubiquitination assay were performed to investigate the interaction between PARK2 and NLRP3. NLRP3 inflammasome activity, IL-1β and IL-18 secretion, pyroptosis, and epithelial barrier integrity were detected to explore the role of PARK2 in allergic asthma. Results: PARK2 expression was remarkably down-regulated in HDM-treated BEAS-2B cells. In BEAS-2B cells, NLRP3 protein was reduced by PARK2 overexpression and increased by PARK2 knockdown. Interestingly, PARK2 overexpression and knockdown didn't affect NLRP3 mRNA. Co-immunoprecipitation assay showed that PARK2 interacted with NLRP3. Proteasome inhibitor MG132 abolished PARK2 overexpression-induced down-regulation of NLRP3 protein. Ubiquitination assays showed that PARK2 overexpression enhanced the ubiquitination of NLRP3. Collectively, PARK2 negatively regulates NLRP3 protein via ubiquitination. In HDM-treated BEAS-2B cells, PARK2 overexpression repressed HDM-induced NLRP3 inflammasome activation, IL-1β and IL-18 secretion, pyroptosis, and epithelial barrier dysfunction. In BEAS-2B cells, PARK2 knockdown promoted NLRP3 inflammasome activation, IL-1β and IL-18 secretion, pyroptosis, and barrier impairment, while its effects were abrogated by NLRP3 inhibitor INF39. Conclusion: Our study demonstrates that PARK2 attenuates HDM-induced NLRP3 inflammasome activation, the release of inflammatory cytokines, pyroptosis, and barrier impairment in airway epithelial cells by ubiquitinating NLRP3.

Keywords: PARK2, NLRP3, inflammation, pyroptosis, airway epithelial barrier dysfunction

Introduction

Asthma is a chronic bronchial condition affecting about 45.7 million people in China, which leads to a great burden of disease [1]. Its common type allergic asthma, which occurs when inhaling allergens like house dust mite (HDM), pollen and PM2.5, can give rise to airflow obstruction and subsequently induce symptoms including wheezing, coughing and chest tightness [2, 3]. Airway epithelial barrier is the first barrier against inhaled allergens and thus plays a critical protective role in maintaining the airway microenvironment [4]. Impaired airway epithelial barrier, mainly originating from cell death and loss of tight junction (TJ) proteins, may cause airway sensitization, hyperresponsiveness, and inflammation [5, 6].

NLRP3 inflammasome is a cytosolic protein complex comprising NLRP3, ASC (PYD and CARD domain containing), and pro-caspase-1 [7]. When NLRP3 inflammasome is activated, pro-caspase-1 is cleaved into the active form and then induces the release of IL-1β and IL-18, and pyroptosis, an inflammatory form of cell death [8]. In many inflammatory diseases, like arthritis, lupus nephritis and allergic asthma, NLRP3 inflammasome is activated and initializes an inflammatory response [9-11]. Recently,

Table 1. Primers for RT-qPCR

Genes	Forward primers (5'-3')	Reverse primers (5'-3')
PARK2	CACGACCCTCAACTTGGCTAC	CGGTTGTACTGCTCTTCTCCC
NLRP3	TTCGGAGATTGTGGTTGGG	GTCACCGAGGGCGTTGTC
GAPDH	AATCCCATCACCATCTTC	AGGCTGTTGTCATACTTC

researchers have found that activated NLRP3 inflammasome also impairs epithelial integrity in enterocytes, cholangiocytes, endotheliocyte and proximal tubular cells [12-15].

PARK2 is an E3 ubiquitin ligase regulating the degradation of many functional proteins, thereby being involved in multiple cellular processes. For example, PARK2 has been found to promote apoptosis in A549 cells by reducing the anti-apoptosis protein Bcl-XL via ubiquitination [16]. PARK2 mediates the mitophagy in HEK293A cells via ubiquitinating the mitochondrial protein BNIP3L [17]. As a Parkinson's disease-associated gene, the mutation of PARK2 occurs frequently in Parkinson's patients [19]. It is also found to be a tumor suppressor in many cancers, like osteosarcoma, lung and pancreatic cancer [20-22]. Nevertheless, whether PARK2 has a role in asthma is unknown.

Here, we used HDM to treat BEAS-2B cells to mimic allergic asthma in vitro. We observed that PARK2 expression was remarkably downregulated in HDM-treated BEAS-2B cells. By searching UbiBrowser database, we found that NLRP3 was a potential substrate of PARK2. In HDM-injured BEAS-2B cells, we further elucidated that PARK2 inhibited HDM-induced NLRP3 inflammasome activation, IL1-β/IL-18 secretion, pyroptosis and barrier dysfunction by ubiquitinating NLRP3.

Materials and methods

Cell culture and treatment

BEAS-2B cells were obtained from the American Type Culture Collection (ATCC, Manassas, USA) and maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma, France) containing 15% fetal bovine serum (FBS, Genetimes, China) with 95% O_2-5% CO_2 at 37°C. Cells were cultured with 300 ng/ml HDM for 24 h to mimic allergic asthma in vitro.

Quantitative real-time PCR (RT-qPCR)

Total RNA in BEAS-2B cells was extracted using Trizol reagent (Sigma, USA), and the residual DNA was cleaned with DNase I. The first-strand cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Canada). RT-qPCR was performed in a Real-Time PCR System (ABI, USA) with SYBR Green mix (Qiagen, Germany). GAPDH was selected as the internal reference, and the relative expression level of mRNA was calculated using $2^{-\Delta\Delta Ct}$ method. The used primers are shown in **Table 1**.

Western blotting

Total protein was obtained from cell lysis solution, and quantified with a BCA protein assay kit (Pierce Chemical, USA). 30 μg protein was split by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes. After incubation with 5% skim milk overnight at 4°C, these membranes were incubated with antibodies against PARK2 (1:1000; Proteintech, USA), NLRP3 (1:1000; Abcam, USA), GSDMD-N (1:1000; Abcam), active caspase1 p20 (1:1000; Proteintech), pro-caspase1 (1:1000; Abcam), ZO-1 (1:1000; Abcam) and GAPDH (1:5000; Proteintech) for 2 h at room temperature. Again, they were incubated with secondary antibody for another 1 h. Finally, the membranes were treated with ECL reagent (Millipore, USA) and visualized in Tanon-5200 system (Shanghai, China).

Lentivirus construction and transduction

Lentivirus oePARK2 and siPARK2 were constructed to overexpress and knock down PARK2 expression, respectively. For the construction of oePARK2, PARK2 gene fragment was inserted into the vector plasmid pLVX-Puro (Clontech, USA), and then the vector plasmid was co-transfected into 293T cells with packaging plasmids psPAX2 and pMD2G (Addgen). 72 h later, the supernatants containing lentiviruses were collected. The process of siPARK2 construction is the same as oePARK2 except that PARK2 siRNA was inserted into the vector plasmid PLKO.1 (Addgene, USA). Three siRNAs of PARK2 are shown as follows: siPARK2-1: 5'-CCAGCAUCUUCCAGCUCAATT-3'; siPARK2-2: 5'-GCAGAGCAUUGUUCACAUUTT-3'; siPARK2-3: 5'-GCAGGUAGAUCAAUCUAUATT-3'. To overexpress and knock down PARK2 in BEAS-2B, 1×10^6 BEAS-2B cells were seeded into each well of a

6-well plate, and then they were transduced with lentiviruses at a multiplicity of infection of 10 at 37°C. 24 h later, the medium was replaced with fresh medium and the cells were cultured for another 24 h.

Measurement of TEER and permeability

3×10^5 Cells were seeded onto Transwell inserts (area 0.6 cm^2, pore size 0.4 μm; Costar, USA) and cultured in DMEM medium for three weeks to allow polarization. Trans-epithelial electrical resistance (TEER) was measured using a Volt-Ohm meter (Millipore, USA). The permeability of cell monolayers was also assessed. In brief, 10 μl of 4 kDa Fluoresceinisothiocyanate (FITC)-dextran (10 mg/ml) was added into the apical side, and 200 μl basolateral medium was collected after 2 h. The fluorescent intensity was detected using a fluorescent microplate reader (Varioskan, Thermo Fisher Scientific, USA) at an excitation/emission wavelength of 490/520 nm.

Co-immunoprecipitation and ubiquitination analyses

For co-immunoprecipitation assay, total protein was incubated with antibodies against IgG, PARK2 or NLRP3 at 4°C overnight, and subsequently incubated with 30 μl Protein A/G-Agarose at 4°C for 2 h. Afterwards, the A/G-Agarose beads were washed 5 times with loading buffer and then boiled for 5 min. The precipitated proteins were collected after centrifugation and then detected by western blott. For ubiquitination analysis, immunoprecipitation and immunoblot analysis were performed using anti-NLRP3 and anti-ubiquitin antibody (1:1000; Sigma-Aldrich, China), respectively.

ELISA assay

After experimental treatment, the culture supernatants of BEAS-2B cells were collected, and the levels of IL-1β and IL-18 were assessed by ELISA kit (Institut Pourquie, France) under the instruction of manufacturer. In brief, 50 μl sample was added into an anti-IL-1β-coated (or anti-IL-18 coated) ELISA plate (R&D Systems, USA) and then incubated for 30 min at 37°C. Next, 50 μl enzyme-labeled antibodies were added for further incubation for 30 min. Afterwards, the plate was treated with 100 μl TMB substrate for 15 min in the dark at 37°C. Finally, the terminal liquid was added to stop the developing. The absorbance was read at 450 nm using a microplate reader (Bio-Rad, USA), and the concentrations of IL-1β and IL-18 were calculated based on the standard curve.

The measurment of LDH activity

The LDH activity in the culture supernatants of BEAS-2B cells was assessed using a LDH Quantification kit (Jining Shiye, Shanghai, China) under the instruction of manufacturer. Briefly, the collected sample was incubated with nicotinamide-adenine dinucleotide and pyruvate for 15 min at 37°C. Then, 0.4 mol/L NaOH was added to stop the reaction. The absorbance was detected at 440 nm using a microplate reader.

Pyroptosis detection

BEAS-2B cells were digested by trypsin-EDTA solution and centrifuged at 1000 rpm for 5 min. Then cells were resuspended in PBS to count. 1×10^5 cells were cultured with 10 μL propidium iodide (PI) and 10 μL caspase-1 (Immunochemistry Technologies, USA) for 30 min in the dark. Finally, Pyroptotic cells were detected using a flow cytometer (BD Biosciences, USA).

Statistics

All experiments were repeated three times. Student's *t* test was performed in Graph-Pad Prism 8.0 to compare the difference between groups. $P < 0.05$ is significant in statistics.

Results

PARK2 expression decreases in HDM-treated BEAS-2B cells

BEAS-2B cells were cultured with 300 ng/ml of HDM to mimic allergic asthma in vitro. PARK2 expression level was detected at 0, 6, 12 and 24 h after HDM treatment. RT-qPCR assay indicated that PARK2 mRNA was obviously decreased in HDM-treated BEAS-2B cells (**Figure 1A**). Western blotting showed that PARK2 protein had a similar downward trend in HDM-treated BEAS-2B cells (**Figure 1B**).

PARK2 negatively regulates NLRP3 protein via ubiquitination

Considering that PARK2 is an E3 ubiquitin ligase, we used UbiBrowser database (http://

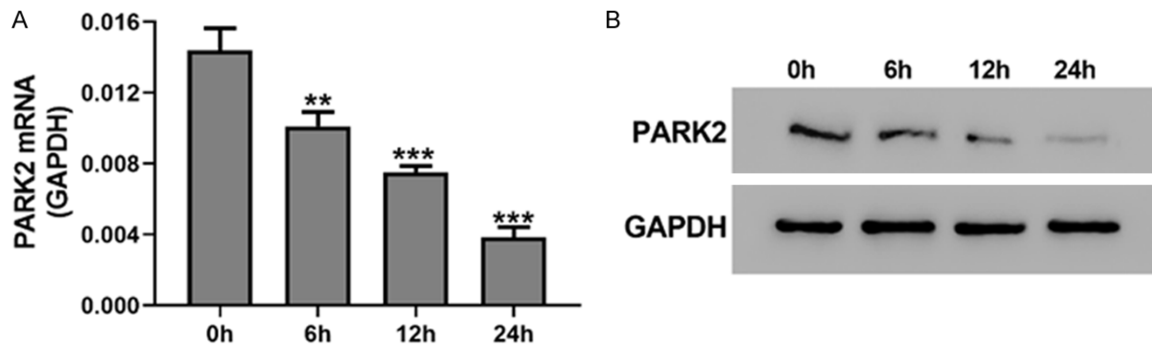

Figure 1. The PARK2 expression was remarkably decreased in HDM-induced BEAS-2B cells. A. PARK2 mRNA was determined by RT-qPCR. B. PARK2 protein was determined by western blot. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs 0 h.

ubibrowser.ncpsb.org/) to search its possible substrates. NLRP3 was predicted to be a potential target of PARK2. We overexpressed and knocked down PARK2 expression in BEAS-2B cells by transducing lentivirus oePARK2 and siPARK2, respectively. Results indicated that PARK2 expression was markedly up-regulated by oePARK2, while it was down-regulated by siPARK2 (**Figures 2A**, **2B**, S1). Conversely, NLRP3 protein was obviously reduced by PARK2 overexpression, whereas it was increased by PARK2 knock-down (**Figure 2B**). Interestingly, both PARK2 overexpression and knock-down didn't affect the mRNA level of NLRP3 (**Figure 2A**). By performing co-immunoprecipitation assay, we found that PARK2 interacted with NLRP3 (**Figure 2C**). In addition, proteasome inhibitor MG132 abolished oePARK2-mediated decrease of NLRP3 protein (**Figure 2D**). Ubiquitination assays further showed that PARK2 overexpression enhanced the ubiquitination of NLRP3 (**Figure 2E**). Collectively, our data indicate that PARK2 reduces NLRP3 protein via ubiquitination.

PARK2 overexpression alleviates HDM-mediated NLRP3 inflammasome activation, IL-1β/IL-18 secretion and pyroptosis

In HDM-treated BEAS-2B cells, NLRP3 and active caspase1 p20 proteins were markedly increased, and the levels of IL-1β and IL-18 in the cell supernatant were dramatically elevated (**Figure 3A**, **3B**). It suggests that HDM induced NLRP3 inflammasome activation and the secretion of IL-1β and IL-18 in BEAS-2B cells. Moreover, HDM treatment up-regulated GSDMD-N protein level, LDH activity and the proportion of PI+/caspase-1+ cells, indicating that HDM also promoted pyroptosis in BEAS-2B cells (**Figure 3A**, **3C**, **3D**). By transducing HDM-treated BEAS-2B cells with oePARK2, we found that all HDM-induced changes were ameliorated by PARK2 overexpression (**Figure 3**). Therefore, PARK2 alleviates HDM-induced NLRP3 inflammasome activation, the secretion of IL-1β and IL-18 secretion, and pyroptosis in BEAS-2B cells.

PARK2 overexpression attenuates HDM-induced airway epithelial barrier dysfunction

We then investigated whether PARK2 affected epithelial barrier function in HDM-injured BEAS-2B cells. TEER value, permeability of FITC-dextran and the expression level of tight junction protein ZO-1 were detected to assess the barrier function of BEAS-2B cells. We found that HDM treatment reduced TEER value and ZO-1 protein while enhancing the permeability of FITC-dextran (**Figure 4**). It indicates that HDM treatment impairs epithelial barrier function in BEAS-2B cells. However, PARK2 overexpression abolished the regulation of HDM on TEER value, permeability of FITC-dextran and ZO-1 protein (**Figure 4**), suggesting that PARK2 suppresses HDM-induced barrier dysfunction in BEAS-2B cells.

PARK2 knock-down induces NLRP3 inflammasome activation, IL-1β/IL-18 secretion, pyroptosis and barrier dysfunction via regulating NLRP3

To verify that PARK2 functioned by regulating NLRP3 protein, BEAS-2B cells were transduced with siPARK2 and then cultured with 10 μM NLRP3 inhibitor INF39. We found that siPARK2 increased the protein levels of NLRP3, active caspase1 p20 and GSDMD-N, promoted IL-1β

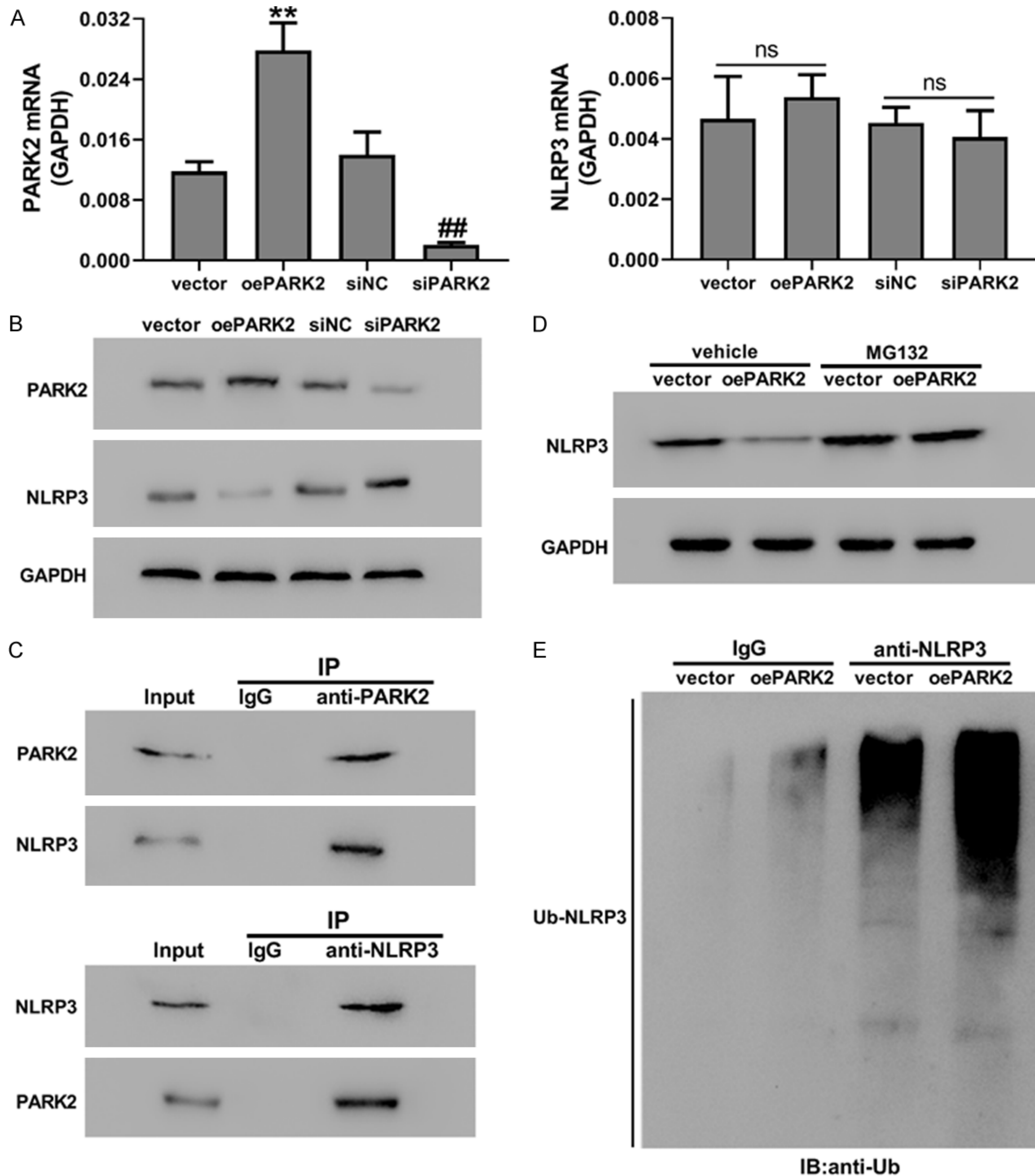

Figure 2. PARK2 negatively regulates NLRP3 by ubiquitination. (A and B) BEAS-2B cells were transduced with oePARK2 or siPARK2. The mRNA (A) and protein (B) levels of PARK2 and NLLRP3 were measured by RT-qPCR and western blot, respectively. (C) The interaction between PARK2 and NLRP3 was detected by co-immunoprecipitation assay. (D) BEAS-2B cells were transduced with oePARK2, and then cultured with 10 umol/L MG132. PARK2 protein level was detected by western blot. (E) BEAS-2B cells were transduced with oePARK2. The ubiquitination level of NLRP3 was determined by ubiquitination assays. $^{**}P < 0.01$ vs vector; $^{\#\#}P < 0.01$ vs siNC. Ns: not significant.

and IL-18 secretion, enhanced LDH activity, and up-regulated the proportion of PI+/caspase-1+ cells (**Figure 5A-D**). These data suggest that PARK2 knockdown activates NLRP3 inflammasome and induces subsequent secretion of IL-1β and IL-18 as well as pyroptosis. In addition, siPARK2 reduced TEER value and ZO-1 protein while enhancing the permeability of FITC-dextran, indicating that PARK2 knockdown impairs the barrier function of BEAS-2B

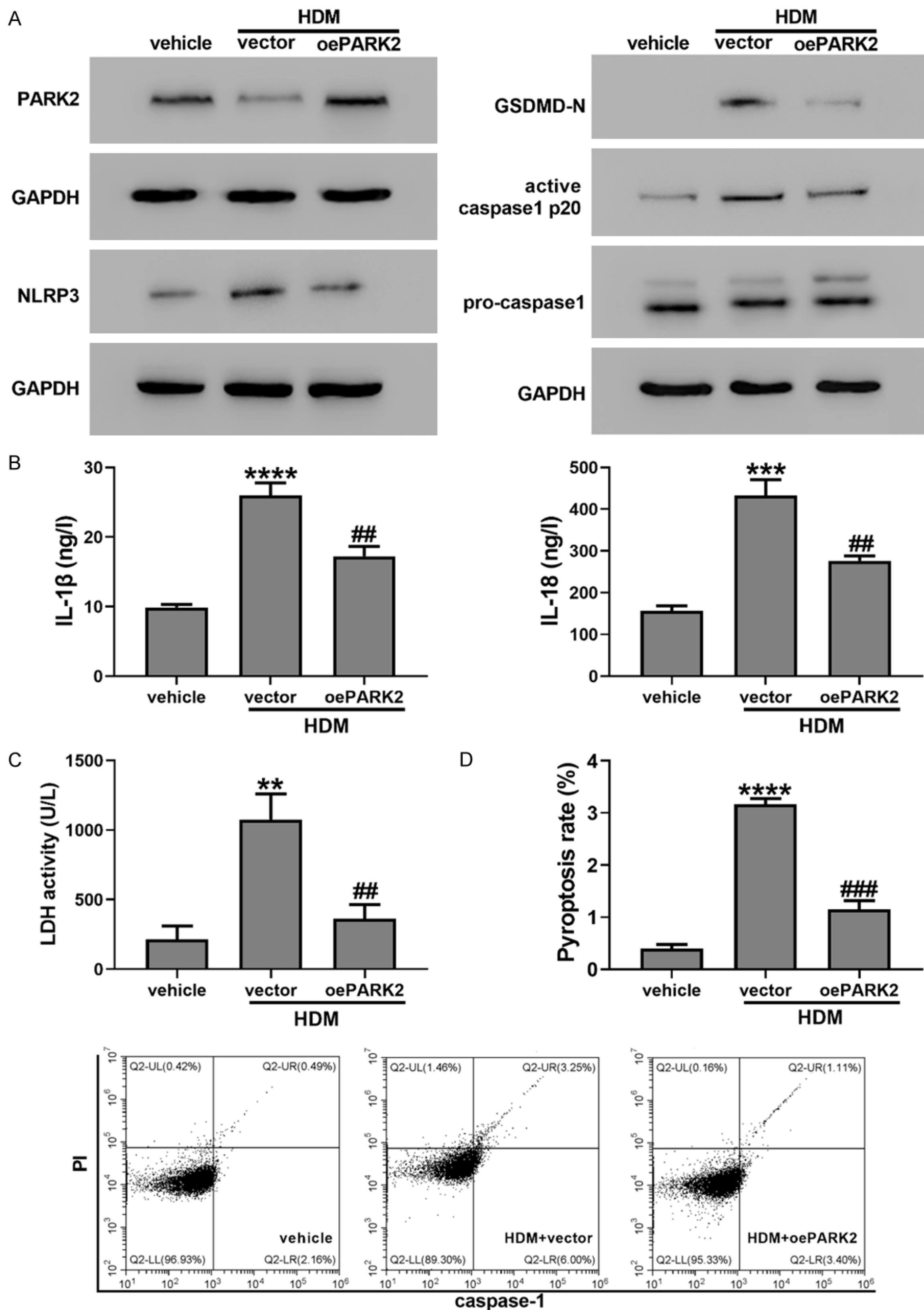

Figure 3. PARK2 overexpression alleviated HDM-induced NLRP3 inflammasome activation, IL-1β/IL-18 secretion and pyroptosis in BEAS-2B cells. Cells were transduced with oePARK2, and then cultured with 300 ng/ml HDM. (A) The protein levels of PARK2, NLRP3, GSDMD-N, active caspase1 p20, pro-caspase1 were detected by western blot. (B and C) The levels of IL-1β and IL-18 (B), and the LDH activity (C) in the cell supernatant were determined by ELISA

and LDH Quantification kit, respectively. (D) The BEAS-2B cells were stained by PI and caspase-1, and then they were detected using a flow cytometer. **P < 0.01, ***P < 0.001 and ****P < 0.0001 vs vehicle; ##P < 0.01 and ###P < 0.001 vs HDM + vector.

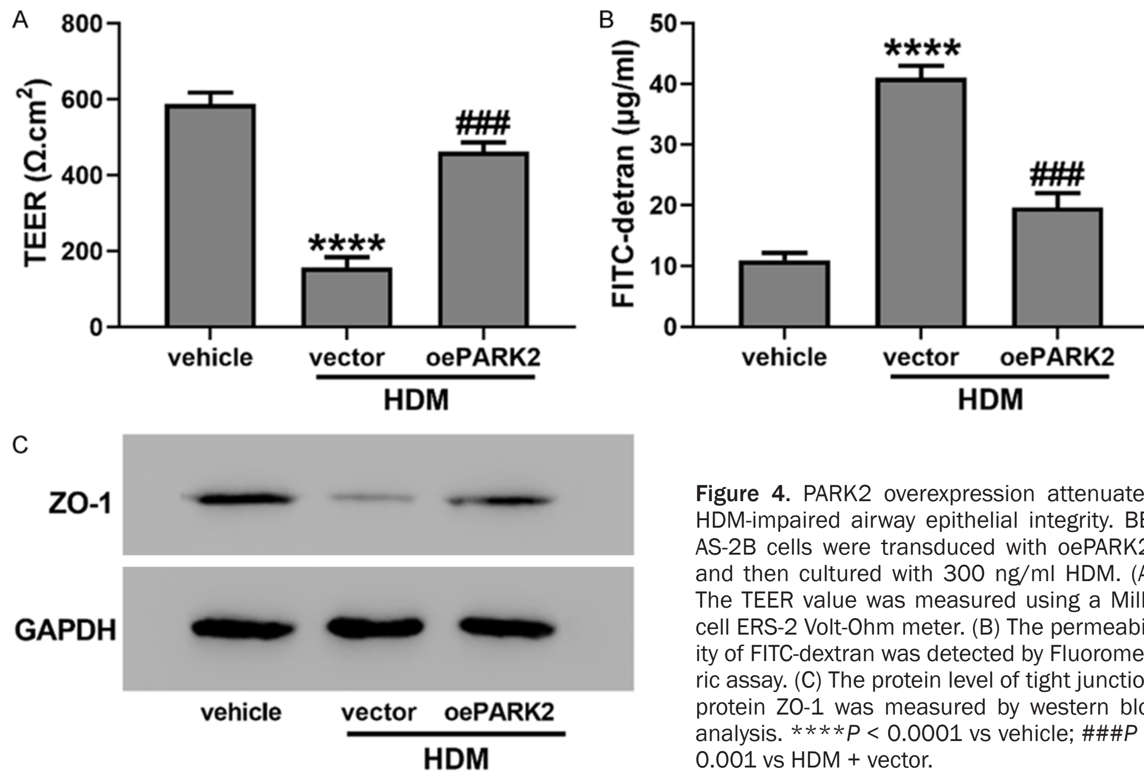

Figure 4. PARK2 overexpression attenuated HDM-impaired airway epithelial integrity. BEAS-2B cells were transduced with oePARK2, and then cultured with 300 ng/ml HDM. (A) The TEER value was measured using a Millicell ERS-2 Volt-Ohm meter. (B) The permeability of FITC-dextran was detected by Fluorometric assay. (C) The protein level of tight junction protein ZO-1 was measured by western blot analysis. ****P < 0.0001 vs vehicle; ###P < 0.001 vs HDM + vector.

cells (**Figure 5A**, **5E**, **5F**). However, all the above effects of PARK2 knockdown were abolished by INF39-induced inhibition of NLRP3 (**Figure 5**). Together, PARK2 knockdown promotes NLRP3 inflammasome activation, IL-1β and IL-18 secretion, pyroptosis and barrier dysfunction via regulating NLRP3.

Discussion

PARK2 is a Parkinson's disease-associated gene whose mutation occurs frequently in Parkinson's patients[23]. In cancer, PARK2 is a tumor suppressor that represses tumorigenesis and enhances apoptosis of cancer cells [20, 21, 24]. However, whether PARK2 has a role in asthma is unknown. In the present study, we observed that PARK2 expression was significantly down-regulated in HDM-treated BEAS-2B cells. By searching UbiBrowser database and performing ubiquitination-related experiments, we found that PARK2 negatively regulates NLRP3 via ubiquitination. In addition, PARK2 overexpression inhibited HDM-mediated NLRP3 inflammasome activation, IL-1β and IL-18 secretion, pyroptosis, and barrier dysfunction. Furthermore, NLRP3 inhibitor INF39 abolished PARK2 knockdown-mediated NLRP3 inflammasome activation, IL-1β and IL-18 secretion, pyroptosis, and barrier dysfunction. Collectively, we conclude that PARK2 protects BEAS-2B cells against HDM-induced injury by ubiquitinating NLRP3, suggesting a possible protective role of PARK2 in HDM-mediated allergic asthma.

Recent studies have noticed the association between PARK2 and inflammatory response. Lee et al. (2016) observed that inflammatory cytokines were increased in PARK2 knockout mice and their elevation was inhibited by exogenous PARK2 expression [25]. In BEAS-2B cells, they further revealed that PARK2 repressed NF-κB activation by regulating ROS/Akt pathway [25]. On the other hand, PARK2 deficiency has been found to aggravate lipopolysaccharide-induced NLRP3 inflammasome activation and the release of IL-1β and IL-18

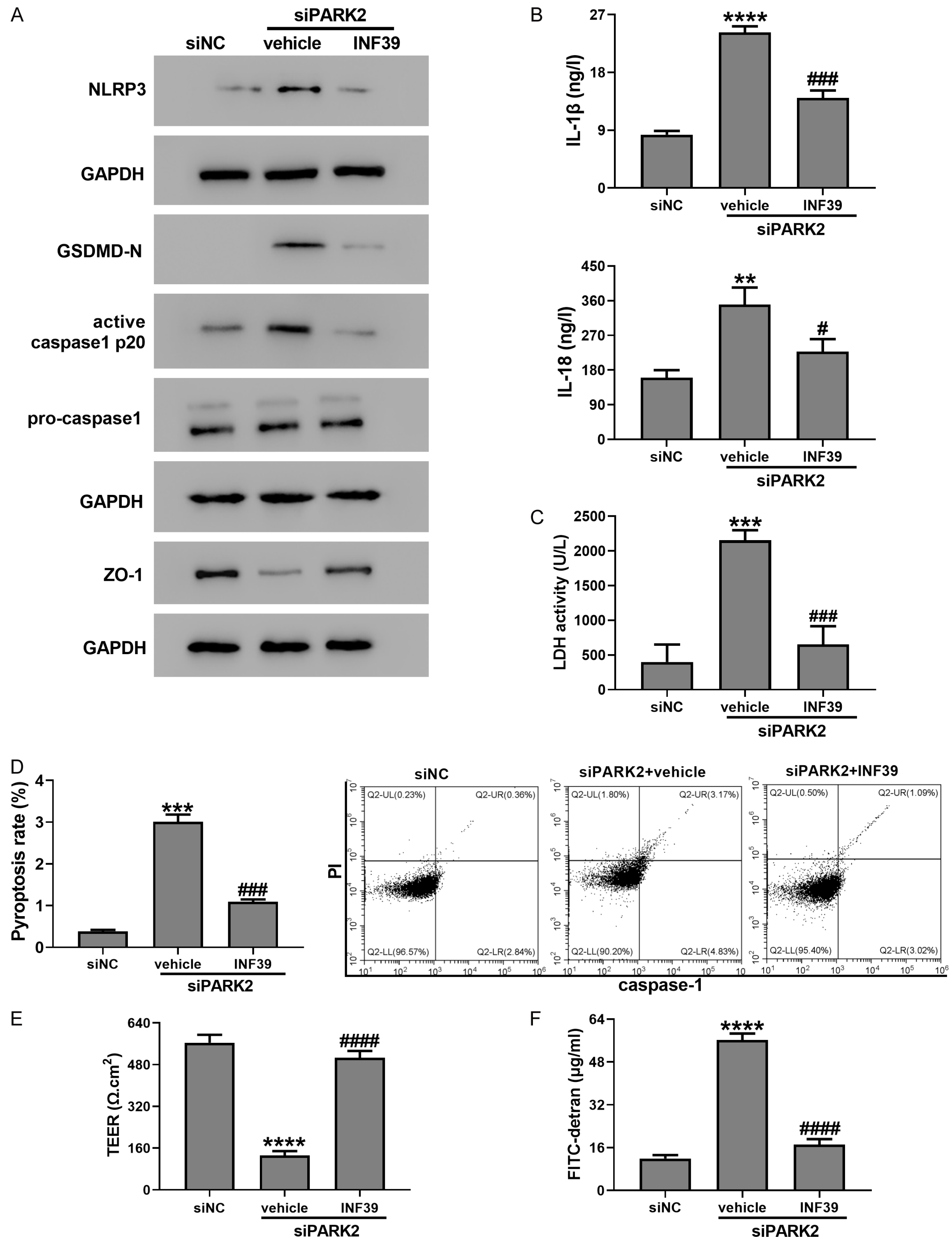

Figure 5. PARK2 knock-down induced NLRP3 inflammasome activation, IL-1β/IL-18 secretion, pyroptosis and barrier dysfunction via regulating NLRP3. BEAS-2B cells were transduced with siPRAK2, and then cultured with 10 μM NLRP3 inhibitor INF39. (A) The protein levels of NLRP3, GSDMD-N, active caspase1 p20, pro-caspase1 and ZO-1 were measured by western blot. (B and C) The levels of IL-1β and IL-18 (B), and the LDH activity (C) were determined by ELISA and LDH Quantification kit, respectively. (D) The BEAS-2B cells were stained by PI and caspase-1, and then they were detected using a flow cytometer. (E) The TEER value was measured using a Millicell ERS-2 Volt-Ohm meter. (F) The permeability of FITC-dextran was detected by Fluorometric assay. $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ vs siNC; $^{\#}p < 0.05$, $^{\#\#\#}P < 0.001$ and $^{\#\#\#\#}P < 0.0001$ vs siPARK2 + vehicle.

[26]. And inhibition of NLRP3 inflammasome ameliorated experimental sepsis in PARK2 knockout mice by reducing the release of IL-1β and HMGB1 [27]. Nevertheless, the detailed relationship between PARK2 and NLRP3 inflammasome is unknown. In the present study, we clearly demonstrate that PARK2 inhibits NLRP3 inflammasome activation and subsequent secretion of IL-1β and IL-18 by ubiquitinating NLRP3. Considering the regulatory effect of PARK2 on NF-κB signaling and NLRP3 inflammasome, we speculate that PARK2 may have an important role in inflammatory disease, which deserves to be explored in the future.

Pyroptosis, an inflammatory programmed cell death, has been found to be implicated in the pathogenesis of asthma. According to the report of Zhuang et al. (2020), airway epithelial pyroptosis is triggered and subsequently exacerbates airway inflammation and hyper-responsiveness in toluene diisocyanate-induced asthmatic mice [28]. Tsai et al. (2018) found that HDM promotes pyroptosis in airway epithelial cells by activating NLRP3 inflammasome [29]. Here, we further uncovered that PARK2 is involved in HDM-induced pyroptosis and is an upstream negatively regulator of NLRP3 inflammasome.

Airway epithelial cells form the first barrier against inhaled harmful substances. Impaired epithelial integrity is the hallmark of allergic asthma [4]. Existing studies have found that NLRP3 inflammasome activation impairs barrier function in many epithelial cell lines, such as intestinal epithelial cells and endotheliocyte [12, 15]. Here, we also found that NLRP3 inflammasome activation is involved in HDM-mediated airway epithelial barrier dysfunction. Cell death and the loss of tight junction proteins are two main causes of epithelial barrier dysfunction [5]. Our data showed HDM simultaneously induces epithelial barrier dysfunction, pyroptosis and the decrease of ZO-1 protein, indicating that the impaired epithelial integrity may be due to pyroptosis and the loss of ZO-1 protein.

In conclusion, our study demonstrates that PARK2 suppresses HDM-induced secretion of IL-1β and IL-18, pyroptosis, and barrier dysfunction in airway epithelial cells by ubiquitinating NLRP3, suggesting a potential protective role of PARK2 in allergic asthma. Further studies are needed to confirm our findings in vivo.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Huang K, Yang T, Xu J, Yang L, Zhao J, Zhang X, Bai C, Kang J, Ran P and Shen H. Prevalence, risk factors, and management of asthma in China: a national cross-sectional study. Lancet 2019; 394: 407-418.

[2] Aaron SD, Boulet LP, Reddel HK and Gershon AS. Underdiagnosis and overdiagnosis of asthma. Am J Respir Crit Care Med 2018; 198: 1012-1020.

[3] Cheng Q, Yang C, Guo B, Wei X and Liu M. Analysis of mechanism of PM2. 5 and house dust mite antigen Der p1 in attack stage of child asthma. Eur Rev Med Pharmacol Sci 2017; 21: 2458-2462.

[4] Post S, Nawijn M, Hackett T, Baranowska M, Gras R, Van Oosterhout A and Heijink I. The composition of house dust mite is critical for mucosal barrier dysfunction and allergic sensitisation. Thorax 2012; 67: 488-495.

[5] Holgate ST. The sentinel role of the airway epithelium in asthma pathogenesis. Immunol Rev 2011; 242: 205-219.

[6] Sweerus K, Lachowicz-Scroggins M, Gordon E, LaFemina M, Huang X, Parikh M, Kanegai C, Fahy JV and Frank JA. Claudin-18 deficiency is associated with airway epithelial barrier dysfunction and asthma. J Allergy Clin Immunol 2017; 139: 72-81.

[7] Yan Y, Jiang W, Liu L, Wang X, Ding C, Tian Z and Zhou R. Dopamine controls systemic in-

flammation through inhibition of NLRP3 inflammasome. Cell 2015; 160: 62-73.

[8] Schmidt RL and Lenz LL. Distinct licensing of IL-18 and IL-1β secretion in response to NLRP3 inflammasome activation. PLoS One 2012; 7: e45186.

[9] Walle LV, Van Opdenbosch N, Jacques P, Fossoul A, Verheugen E, Vogel P, Beyaert R, Elewaut D, Kanneganti TD and van Loo G. Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. Nature 2014; 512: 69-73.

[10] Tsai PY, Ka SM, Chang JM, Chen HC, Shui HA, Li CY, Hua KF, Chang WL, Huang JJ and Yang SS. Epigallocatechin-3-gallate prevents lupus nephritis development in mice via enhancing the Nrf2 antioxidant pathway and inhibiting NLRP3 inflammasome activation. Free Radic Biol Med 2011; 51: 744-754.

[11] Kim SR, Kim D, Kim S, Lee H, Lee KS, Cho SH and Lee YC. NLRP3 inflammasome activation by mitochondrial ROS in bronchial epithelial cells is required for allergic inflammation. Cell Death Dis 2014; 5: e1498.

[12] Feng Y, Wang Y, Wang P, Huang Y and Wang F. Short-chain fatty acids manifest stimulative and protective effects on intestinal barrier function through the inhibition of NLRP3 inflammasome and autophagy. Cell Physiol Biochem 2018; 49: 190-205.

[13] Maroni L, Agostinelli L, Saccomanno S, Pinto C, Giordano DM, Rychlicki C, De Minicis S, Trozzi L, Banales JM and Melum E. Nlrp3 activation induces Il-18 synthesis and affects the epithelial barrier function in reactive cholangiocytes. Am J Pathol 2017; 187: 366-376.

[14] Zhuang Y, Hu C, Ding G, Zhang Y, Huang S, Jia Z and Zhang A. Albumin impairs renal tubular tight junctions via targeting the NLRP3 inflammasome. Am J Physiol Renal Physiol 2015; 308: F1012-F1019.

[15] Zhang Y, Chen Y, Zhang YZ, Li PL and Li X. Contribution of cathepsin B-dependent Nlrp3 inflammasome activation to nicotine-induced endothelial barrier dysfunction. Eur J Pharmacol 2019; 865: 172795.

[16] Gong Y, Schumacher SE, Wu WH, Tang F, Beroukhim R and Chan TA. Pan-cancer analysis links PARK2 to BCL-XL-dependent control of apoptosis. Neoplasia 2017; 19: 75-83.

[17] Gao F, Chen D, Si J, Hu Q, Qin Z, Fang M and Wang G. The mitochondrial protein BNIP3L is the substrate of PARK2 and mediates mitophagy in PINK1/PARK2 pathway. Hum Mol Genet 2015; 24: 2528-2538.

[18] Ashrafi G and Schwarz TL. PINK1-and PARK2-mediated local mitophagy in distal neuronal axons. Autophagy 2015; 11: 187-189.

[19] Lücking CB, Dürr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Denèfle P and Wood NW. Association between early-onset Parkinson's disease and mutations in the parkin gene. N Engl J Med 2000; 342: 1560-1567.

[20] Lei Z, Duan H, Zhao T, Zhang Y, Li G, Meng J, Zhang S and Yan W. PARK2 inhibits osteosarcoma cell growth through the JAK2/STAT3/VEGF signaling pathway. Cell Death Dis 2018; 9: 1-13.

[21] Duan H, Lei Z, Xu F, Pan T, Lu D, Ding P, Zhu C, Pan C and Zhang S. PARK2 suppresses proliferation and tumorigenicity in non-small cell lung cancer. Front Oncol 2019; 9: 790.

[22] Li C, Zhang Y, Cheng X, Yuan H, Zhu S, Liu J, Wen Q, Xie Y, Liu J and Kroemer G. PINK1 and PARK2 suppress pancreatic tumorigenesis through control of mitochondrial iron-mediated immunometabolism. Dev Cell 2018; 46: 441-455.

[23] Kilarski LL, Pearson JP, Newsway V, Majounie E, Knipe MDW, Misbahuddin A, Chinnery PF, Burn DJ, Clarke CE and Marion MH. Systematic review and UK-based study of PARK2 (parkin), PINK1, PARK7 (DJ-1) and LRRK2 in early-onset Parkinson's disease. Mov Disord 2012; 27: 1522-1529.

[24] Lee KH, Lee MH, Kang YW, Rhee KJ, Kim TU and Kim YS. Parkin induces apoptotic cell death in TNF-α-treated cervical cancer cells. BMB Rep 2012; 45: 526-531.

[25] Lee S, She J, Deng B, Kim J, De Andrade M, Na J, Sun Z, Wampfler JA, Cunningham JM and Wu Y. Multiple-level validation identifies PARK2 in the development of lung cancer and chronic obstructive pulmonary disease. Oncotarget 2016; 7: 44211.

[26] Mouton-Liger F, Rosazza T, Sepulveda-Diaz J, Ieang A, Hassoun SM, Claire E, Mangone G, Brice A, Michel PP and Corvol JC. Parkin deficiency modulates NLRP 3 inflammasome activation by attenuating an A 20-dependent negative feedback loop. Glia 2018; 66: 1736-1751.

[27] Kang R, Zeng L, Xie Y, Yan Z, Zhou B, Cao L, Klionsky DJ, Tracey KJ, Li J and Wang H. A novel PINK1-and PARK2-dependent protective neuroimmune pathway in lethal sepsis. Autophagy 2016; 12: 2374-2385.

[28] Zhuang J, Cui H, Zhuang L, Zhai Z, Yang F, Luo G, He J, Zhao H, Zhao W and He Y. Bronchial epithelial pyroptosis promotes airway inflammation in a murine model of toluene diisocyanate-induced asthma. Biomed Pharmacother 2020; 125: 109925.

[29] Tsai YM, Chiang KH, Hung JY, Chang WA, Lin HP, Shieh JM, Chong IW and Hsu YL. Der f1 induces pyroptosis in human bronchial epithelia via the NLRP3 inflammasome. Int J Mol Med 2018; 41: 757-764.

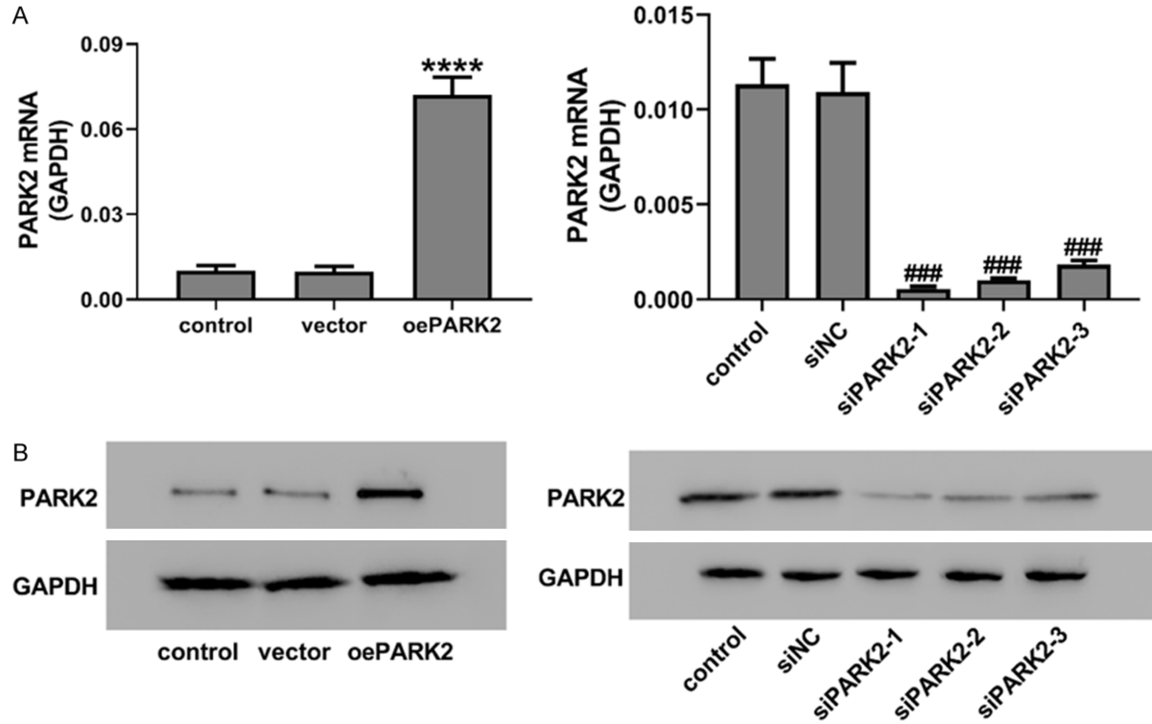

Figure S1. The effects of oePARK2 and siPARK2 on the expression level of PARK2 in BEAS-2B cells. (A and B) Cells were transduced with oePARK2 or siPARK2. After 24 h, PARK2 mRNA (A) and protein (B) levels were detected by RT-qPCR and western blotting, respectively. ****P < 0.0001 vs vector; ###P < 0.001 vs siNC.