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Ubiquitin-specific protease 8 inhibits lipopolysaccharide-triggered pyroptosis of human bronchial epithelial cells by regulating PI3K/AKT and NF- κ B pathways

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NF- κ B

Abstract

Asthma, characterized by dysfunction of airway epithelial cells, is regarded as a chronic inflammatory disorder in the airway. Ubiquitin-specific protease 8 (USP8) belongs to ubiquitin proteasome system and mediates the stability of E3 ligases. The anti-inflammatory effect of USP8 has been widely investigated in distinct diseases, while the role of USP8 in asthma remains elusive. Firstly, human bronchial epithelial cells (BEAS-2B) were treated with lipopolysaccharide, which reduced the cell viability of BEAS-2B and induced the secretion of lactate dehydrogenase (LDH). Moreover, the expression of USP8 was downregulated in BEAS-2B post lipopolysaccharide treatment. Secondly, overexpression of USP8 enhanced cell viability of lipopolysaccharide-treated BEAS-2B, and reduced the LDH secretion. USP8 overexpression also attenuated lipopolysaccharide-induced upregulation of TNF- α , IL-6, and IL-1 β in BEAS-2B. Thirdly, lipopolysaccharide treatment promoted the expression of NLRP3 (NLR Family Pyrin Domain Containing 3), N-terminal domain of gasdermin D (GSDMD-N), caspase-1, IL-1 β , and IL-18 in BEAS-2B, which was inhibited by USP8 overexpression. Lastly, USP8 overexpression decreased the phosphorylation of NF- κ B, while it increased the phosphorylation of PI3K and AKT in lipopolysaccharide-treated BEAS-2B. In conclusion, USP8 inhibited lipopolysaccharide-triggered inflammation and pyroptosis in human bronchial epithelial cells by activating PI3K/AKT signaling and inhibiting NF- κ B signaling pathway.

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Introduction

Asthma, one of the most common chronic respiratory diseases,^{1,2} is a major public health problem among both adults and children with high morbidity and mortality. It is generally characterized by mucus hypersecretion and chronic airway inflammation,³ and is regarded as a global health problem, which has no effective treatment.⁴ Therefore, novel strategies are urgently needed for the prevention of asthma.

Exogenous toxic factors, including smoking, air pollutants, and bacterial infections, can induce viral diseases, such as bronchiolitis and lung injury, and lead to the occurrence of asthma.⁵ Airway epithelium is the first line of defense against those toxic factors.⁵ Lipopolysaccharide, the bioactive ingredient in cigarette smoke and the main component of gram-negative bacteria, has been shown to induce airway inflammation and lead to damage and dysfunction of airway epithelial cells, thus being implicated in the pathogenesis of respiratory diseases, such as asthma.⁶ Suppression of lipopolysaccharide-induced airway inflammation contributed to the amelioration of asthma.⁷

Ubiquitin-specific protease 8 (USP8) is a member of the ubiquitin proteasome system, which functions as a deubiquitinating enzyme to mediate the stability of E3 ligases and exerts pleiotropic functions in endosomal sorting, auto-/mitophagy, neurological disorders, ciliogenesis, sperm acrosome formation, and T-cell function.⁸ It has been shown that USP8 was able to inhibit the activation of microglia, suppress inflammatory response, and improve cognitive and motor impairment, exerting a protective role against sepsis-associated encephalopathy.⁹ USP8 also reduced intermittent hypoxia/reoxygenation-induced inflammation in renal tubular epithelial cells through deubiquitination of transforming growth factor- β -activated kinase-1.¹⁰ Lipopolysaccharide-induced hippocampal damage, inflammatory response, and cognitive and motor deficits in mice were also prevented by USP8 through inhibition of TLR4/MyD88/NF- κ B signaling.¹¹ In addition, analysis of gene expression profiling from asthmatic and nonasthmatic participants showed that USP8 was dysregulated in asthmatic patients.¹² NF- κ B signaling was activated in response to the stimuli of lipopolysaccharide during the development of asthma.¹³ Inhibition of NF- κ B signaling has been shown to be a promising strategy for the interference of asthma.¹³ Therefore, USP8 was hypothesized to treat asthma through inactivation of NF- κ B signaling.

In this study, the effects of USP on lipopolysaccharide-induced cellular damage and inflammation in human bronchial epithelial cells were investigated. The results might provide potential strategy for the treatment of asthma.

Materials and Methods

Cell culture and treatment

Human bronchial epithelial cells (BEAS-2B) were acquired from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum and 1% antibiotics of penicillin-streptomycin. Lipopolysaccharide-induced bronchial epithelial cells have been widely used as an *in vitro* cell model of asthma.¹⁴⁻¹⁶ Cells with at least 80% confluence were incubated with 1, 5, 10, or 20 μ M lipopolysaccharide (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. All the cells were cultured in an incubator at 37°C.

Cell viability

BEAS-2B (3×10^3 cells/well) was seeded in a 96-well plate and incubated with the lipopolysaccharide for 24 h. pcDNA3.1-USP8 (USP8) or negative control (NC) was acquired from Genepharma (Shanghai, China). Cells were then transfected with the vectors using Lipofectamine 2000 (Sigma-Aldrich) for another 24 h. MTT solution (Beyotime, Beijing, China) was then added into each well, and cells were cultured at 37°C for 4 h. Absorbance at 450 nm was measured by microplate reader (Bio-Rad, Hercules, CA, USA), followed by incubation with dimethyl sulfoxide.

qRT-PCR

BEAS-2B with indicated treatment and transfections was lysed with the TRIzol kit (Invitrogen, Carlsbad, CA, USA), and the RNAs were then isolated from the cells. RNAs were synthesized into cDNAs using the Multiscribe™ Reverse transcription Kit (Applied Biosystems, CA, USA), and the mRNA expressions of USP8, TNF- α , IL-6, and IL-1 β were detected by PreTaq II kit (Takara, Dalian, Liaoning, China) with the primers in Table 1. The mRNA expression was normalized to GAPDH through the $2^{-\Delta\Delta Ct}$ method.

Table 1 Primer sequences.

Gene	Forward	Reverse
TNF- α	5'-CATCTTCTCAAAATTCGAGTGACAA-3'	5'-TGGGAGTAGACAAGGTACAACCC-3'
USP8	5'-GCCTGCTACAAAGAGTGTTCCAC-3'	5'-GGAGAGGGAAATACTGGCTTGG-3'
IL-6	5'-GGTGAGTGGCTGTCTGTGTG-3'	5'-TTCGGTCCAGTTGCCTTCT-3'
IL-1 β	5'-TGGACCTTCCAGGATGAGGACA-3'	5'-GTTTCATCTCGGAGCCTGTAGTG-3'
IL-4	5'-GAATGTACCAGGAGCCATATC-3'	5'-CTCAGTACTACGAGTAATCCA-3'
IFN- γ	5'-AACGCTACACACTGCATCTTGG-3'	5'-GACTTCAAAGAGTCTGAGG-3'
GAPDH	5'-GGCATGGACTGTGGTCATGAG-3'	5'-TGCACCCCAACTGCTTAGC-3'

ELISA

The cultured medium of BEAS-2B was harvested, and the protein concentration was measured using the BCA kit (Applygen, Beijing, China). Levels of LDH, TNF- α , IL-6, IL-4, IFN- γ , and IL-1 β were determined using ELISA kits (ExCell Biology, Inc., Shanghai, China).

Western blot

BEAS-2B was lysed in RIPA buffer (Beyotime), and the concentration of isolated proteins was then determined. Samples (30 μ g) were separated by 10% SDS-PAGE, and transferred onto nitrocellulose membranes. The membranes were blocked in 5% BCA, and then probed with specific rabbit-anti-human antibodies: anti-mTOR (ab2732) and anti-p-mTOR (ab1093) (1:1500); anti-IL18 (ab191152), anti-USP8 (ab228572), and anti- β -actin (ab8227) (1:2000); anti-NLRP3 (ab214185) and anti-GSDMD-N (ab155233) (1:2500); anti-caspase-1 (ab62698) and anti-IL-1 β (ab9722) (1:3000); anti-PI3K (ab154598) and anti-p-PI3K (ab182651) (1:3500); anti-p-AKT (ab81283) and anti-AKT (ab8805) (1:4000); and anti-p-NF- κ B (ab28856) and anti-NF- κ B (ab16502) (1:4500). The membranes were probed with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (ab6721) (1:5000), and incubated with enhanced chemiluminescence (Sigma-Aldrich) to determine the immunoreactivities. All the antibodies were purchased from Abcam (Cambridge, UK). Densitometric analysis was calculated using ImageJ 1.51k software.

Statistical analysis

All the data with at least triple replicates were expressed as mean \pm SEM, and analyzed by student's *t*-test or one-way analysis of variance using SPSS software. A *p* value of < 0.05 was considered as statistically significant.

Results

The expression of USP8 was downregulated in lipopolysaccharide-treated BEAS-2B

To mimic the cell model of asthma, human bronchial epithelial cells (BEAS-2B) were incubated with different concentrations of lipopolysaccharide. The mRNA expression of USP8 was downregulated in BEAS-2B by lipopolysaccharide in a dosage dependent way (Figure 1A). Moreover, protein expression of USP8 was also reduced in lipopolysaccharide-treated BEAS-2B (Figure 1B).

USP8 enhanced the cell viability of lipopolysaccharide-treated BEAS-2B

Lipopolysaccharide-treated BEAS-2B was transfected with pcDNA-USP8 (Figure 2A) to investigate the role of USP8 in lipopolysaccharide-treated BEAS-2B. Lipopolysaccharide treatment decreased the cell viability of BEAS-2B

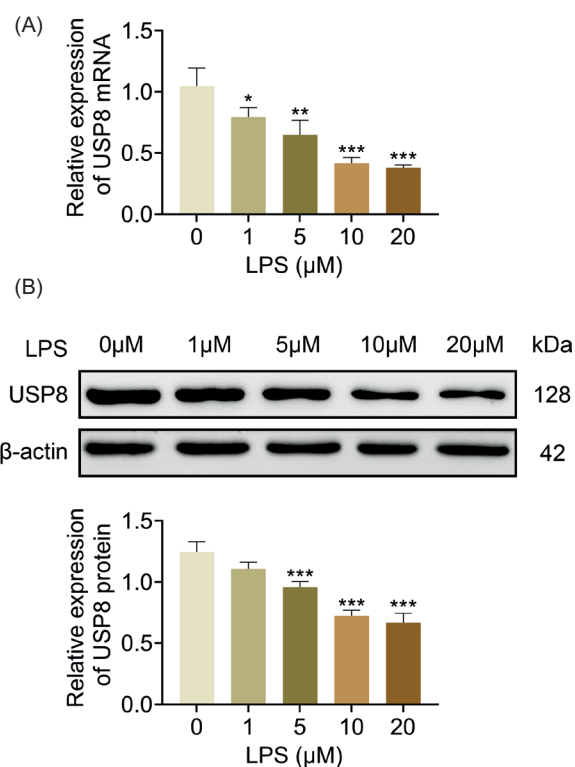


Figure 1 The expression of USP8 was downregulated in lipopolysaccharide-treated BEAS-2B. (A) mRNA expression of USP8 was downregulated in lipopolysaccharide-treated BEAS-2B in a dosage dependent way. (B) Protein expression of USP8 was downregulated in lipopolysaccharide-treated BEAS-2B in a dosage dependent way and detected by western blot. *, **, ***, versus BEAS-2B $p < 0.05$, $p < 0.01$, $p < 0.001$.

(Figure 2B), which was attenuated by the transfection of pcDNA-USP8 into BEAS-2B (Figure 2B). Lipopolysaccharide-induced secretion of LDH in BEAS-2B was inhibited by USP8 transfection (Figure 2C), suggesting the proliferative effect of USP8 in lipopolysaccharide-treated BEAS-2B.

USP8 reduced the inflammatory response of lipopolysaccharide-treated BEAS-2B

Lipopolysaccharide treatment upregulated the levels of TNF- α , IL-6, IL-1 β , IL-4, and IFN- γ in BEAS-2B (Figure 3A and B). However, overexpression of USP8 decreased the levels of TNF- α , IL-6, IL-1 β , IL-4, and IFN- γ in lipopolysaccharide-treated BEAS-2B (Figure 3A and B), demonstrating the anti-inflammatory effect of USP8 in lipopolysaccharide-treated BEAS-2B.

USP8 suppressed pyroptosis of lipopolysaccharide-treated BEAS-2B

Lipopolysaccharide induced upregulation of NLRP3, GSDMD-N, caspase-1, IL-1 β , and IL-18 in BEAS-2B (Figure 4), suggesting that lipopolysaccharide promoted pyroptosis of BEAS-2B.

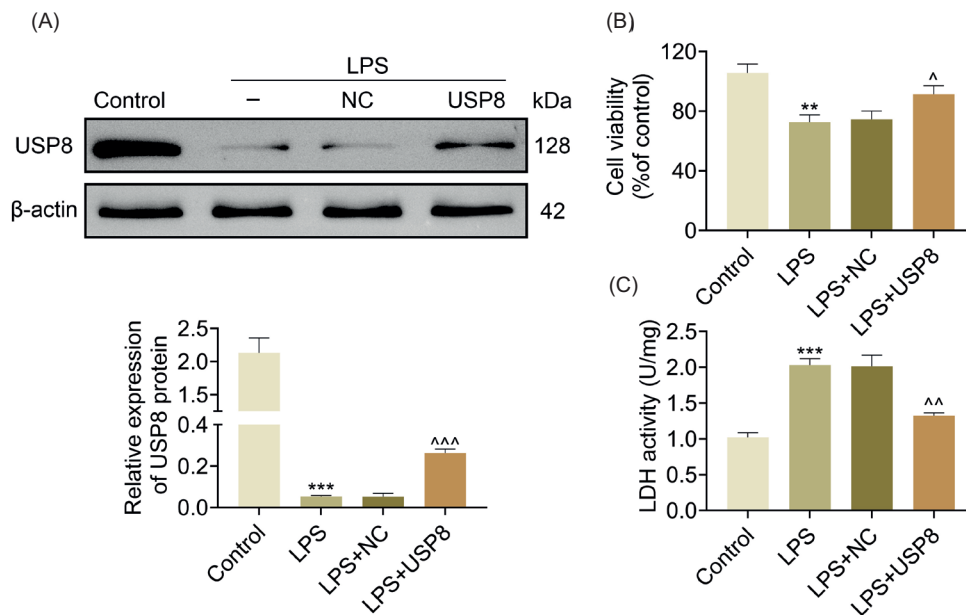


Figure 2 USP8 enhanced cell viability of lipopolysaccharide-treated BEAS-2B. (A) Lipopolysaccharide-treated BEAS-2B was transfected with pcDNA-USP8 to increase the protein expression of USP8 detected by western blot. (B) Transfection of pcDNA-USP8 enhanced the cell viability of lipopolysaccharide-treated BEAS-2B. (C) Transfection of pcDNA-USP8 reduced LDH secretion of lipopolysaccharide-treated BEAS-2B detected by ELISA. **, *** versus control $p < 0.01$, $p < 0.001$. ^, ^^ versus LPS + NC $p < 0.05$, $p < 0.01$.

However, overexpression of USP8 suppressed pyroptosis of lipopolysaccharide-treated BEAS-2B through downregulation of NLRP3, GSDMD-N, caspase-1, IL-1 β , and IL-18 (Figure 4).

USP8 regulated PI3K/AKT and NF- κ B pathways in lipopolysaccharide-treated BEAS-2B

Lipopolysaccharide reduced the phosphorylation of PI3K, AKT, and mTOR in BEAS-2B (Figure 5A), which was reversed by overexpression of USP8 in lipopolysaccharide-treated BEAS-2B (Figure 5A). Moreover, overexpression of USP8 attenuated lipopolysaccharide-induced increase of NF- κ B phosphorylation in BEAS-2B (Figure 5B). These results revealed that USP8 increased the phosphorylation of PI3K, AKT, and mTOR, and inhibited the phosphorylation NF- κ B in lipopolysaccharide-treated BEAS-2B.

Discussion

Ubiquitin proteasome system is essential for protein homeostasis and has been shown to be implicated in the pathogenesis of pulmonary disease.¹⁷ Inhibition of ubiquitin proteasome system promoted the blockage of NF- κ B signaling and suppressed airway inflammation to ameliorate asthma.¹⁸ For example, USP38 contributed to the stabilization of JunB protein to promote asthma pathogenesis.¹⁹ Proteins involved in the ubiquitin proteasome system, including USP10, USP36, USP40, USP34, USP24, and USP8, were dysregulated in asthmatic patients.¹² This study found that overexpression of USP8 attenuated lipopolysaccharide-induced cytotoxicity and inflammation in BEAS-2B, thus ameliorating asthma.

Previous studies have shown that lipopolysaccharide induced asthmatic conditions in human bronchial epithelial cell through promotion of cell insult and inflammation.¹⁵ Therefore, lipopolysaccharide-evoked bronchial epithelial cells were widely used as an asthmatic cell model.¹⁵ Here, we also incubated BEAS-2B with lipopolysaccharide to mimic asthma. USP8 was found to be downregulated in lipopolysaccharide-treated BEAS-2B. Overexpression of USP8 attenuated lipopolysaccharide-evoked cytotoxicity in BEAS-2B through increasing cell viability and decreasing LDH secretion. However, the role of USP8 in cell apoptosis of lipopolysaccharide-evoked BEAS-2B should be investigated in further research.

Airway inflammation, driven by respiratory infections, environmental allergens, and irritants, has been shown to be the main contributor for the development of bronchial obstruction and asthma severity.²⁰ Lipopolysaccharide induced inflammation in bronchial epithelial cells, and inhibition of lipopolysaccharide-induced airway inflammation was regarded as a promising strategy against asthma.¹⁵ USP8 attenuated lipopolysaccharide-induced neuroinflammation and protected against neurodegenerative diseases.¹¹ Here, our results showed that overexpression of USP8 reduced the levels of TNF- α , IL-6, IL-1 β , IL-4, and IFN- γ in lipopolysaccharide-treated BEAS-2B to suppress the airway inflammation. NF- κ B pathway has been shown to regulate cell proliferation and apoptosis of airway smooth muscle cells in asthma,²¹ and blockade of NF- κ B pathway reduced ovalbumin-induced airway inflammation in the rat model of asthma.²² USP8 interacted with transforming growth factor- β -activated kinase-1, deubiquitinated the K63-linked ubiquitination, and promoted the phosphorylation of NF- κ B inhibitor, IKK β , to facilitate the degradation and inactivation of NF- κ B.¹⁰ Results in this study demonstrated that

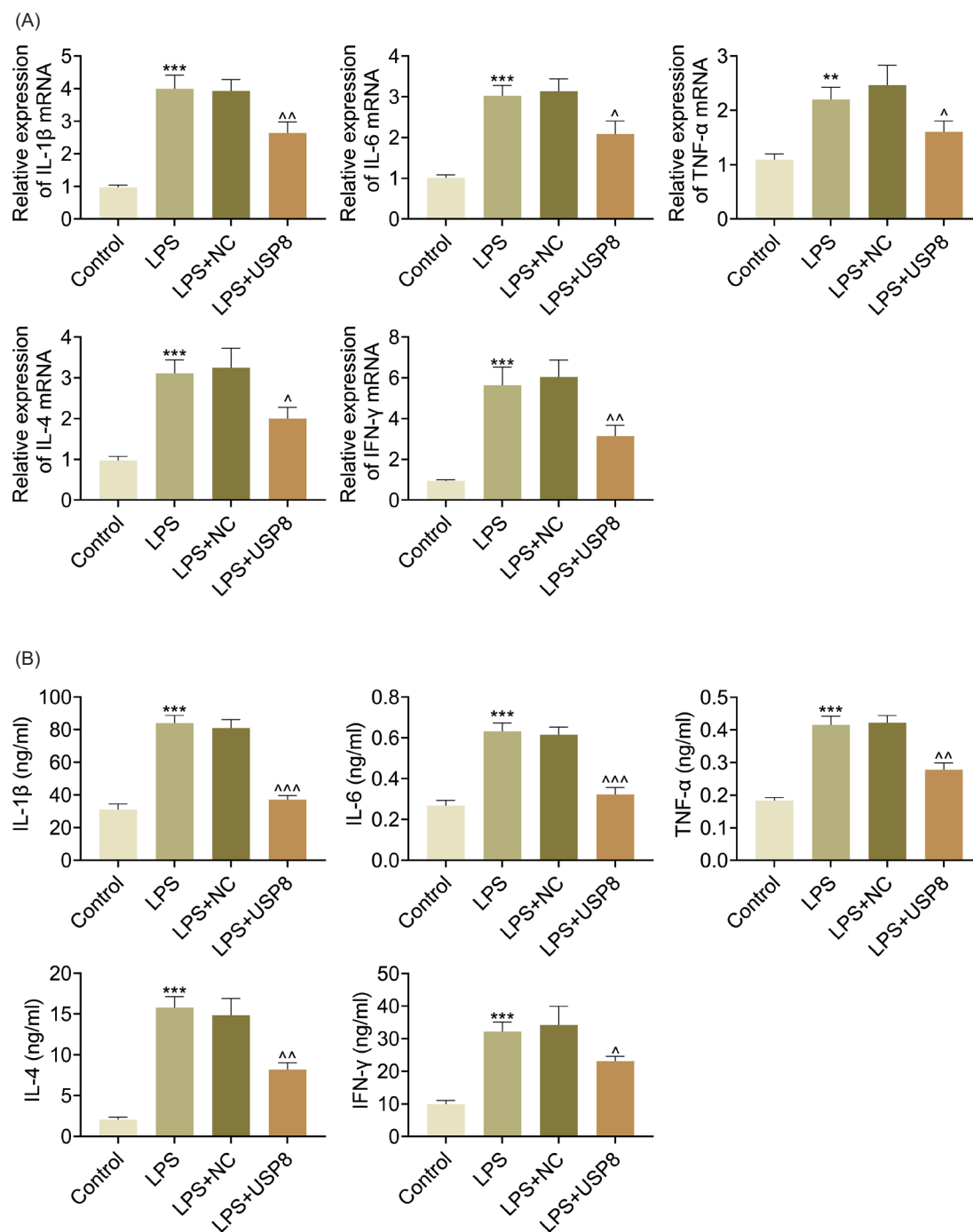


Figure 3 USP8 inhibited inflammatory response in lipopolysaccharide-treated BEAS-2B. (A) Overexpression of USP8 reduced the mRNA expression of TNF- α , IL-6, IL-1 β , IL-4, and IFN- γ in lipopolysaccharide-treated BEAS-2B. (B) Overexpression of USP8 reduced the protein expression of TNF- α , IL-6, IL-1 β , IL-4, and IFN- γ in lipopolysaccharide-treated BEAS-2B detected by ELISA. **, *** versus control $p < 0.01$, $p < 0.001$. ^, ^^, ^^ versus LPS + NC $p < 0.05$, $p < 0.01$, $p < 0.001$.

overexpression of USP8 inhibited lipopolysaccharide-induced increase of NF- κ B phosphorylation in BEAS-2B, thus suppressing activation of NF- κ B signaling in order to exert a protective role against airway inflammation in asthma.

Pyroptosis is a programmed cell death mediated by the activation of a member of the caspase family, namely, caspase-1.²³ Pyroptosis is implicated in respiratory diseases, including asthma, through the promotion of excessive cell

death and inflammation.²⁴ Lipopolysaccharide induced pyroptosis of bronchial epithelial cells,²⁵ and suppression of lipopolysaccharide-induced pyroptosis through downregulation of GSDMD-N, caspase-1, IL-1 β , and IL-18, and contributed to the anti-inflammatory effect against asthma.²⁵ Downregulation of USP8 promoted the upregulation of the markers of pyroptosis, such as NLRP3, pro-caspase-1, cleaved Caspase-1, cleaved GSDMD-N, IL-1 β , and IL-18, thus

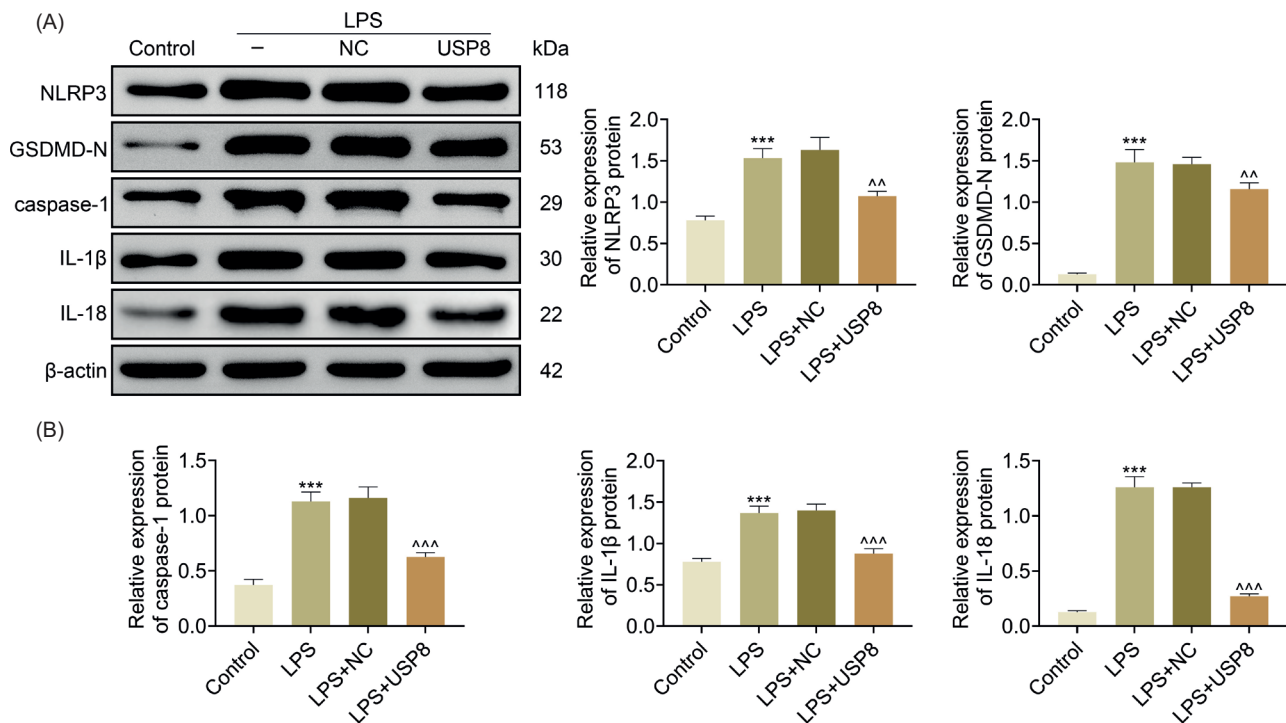


Figure 4 USP8 suppressed the pyroptosis of lipopolysaccharide-treated BEAS-2B. Overexpression of USP8 downregulated the protein expression of NLRP3, GSDMD-N, caspase-1, IL-1 β , and IL-18 in lipopolysaccharide-treated BEAS-2B detected by western blot. *** versus control $p < 0.001$. ^^, ^^^\ versus LPS + NC $p < 0.01$, $p < 0.001$.

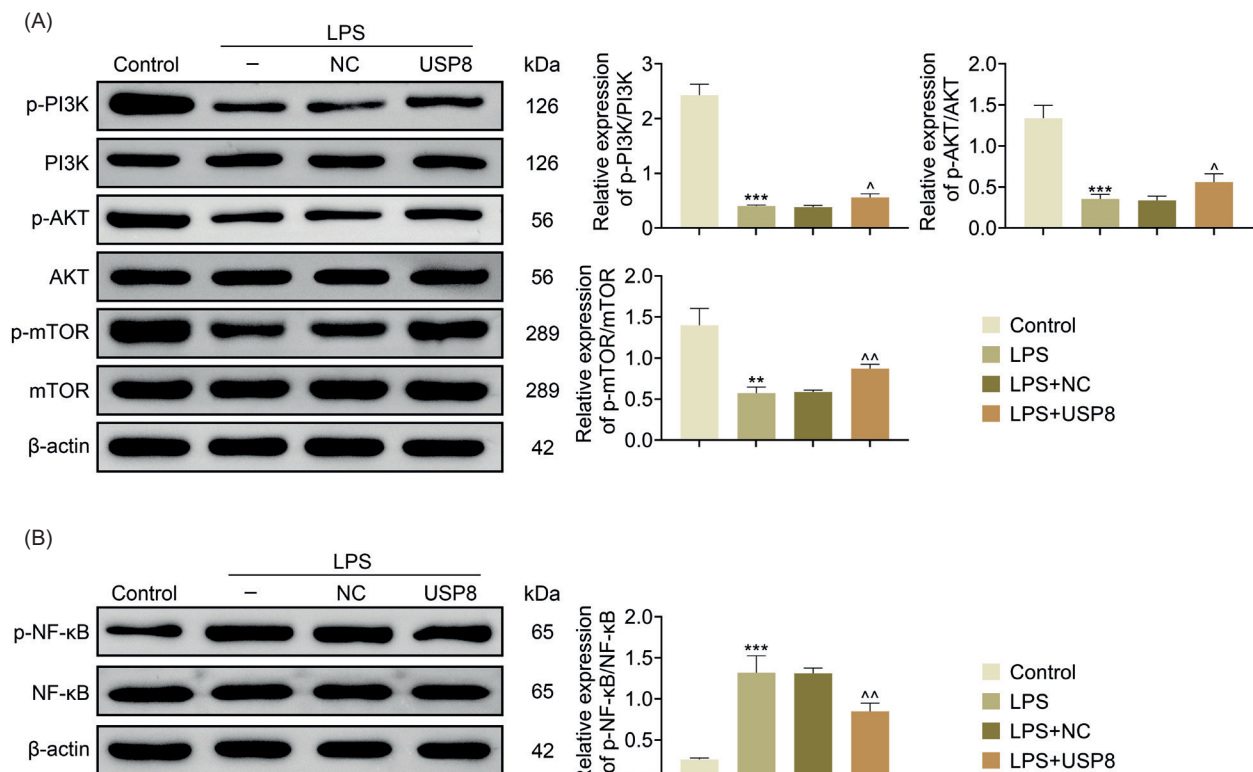


Figure 5 USP8 regulated PI3K/AKT and NF- κ B pathways in lipopolysaccharide-treated BEAS-2B. (A) Overexpression of USP8 enhanced the phosphorylation of PI3K, AKT, and mTOR in lipopolysaccharide-treated BEAS-2B detected by western blot. (B) Overexpression of USP8 reduced the phosphorylation of NF- κ B in lipopolysaccharide-treated BEAS-2B detected by western blot. *** versus control $p < 0.001$. ^, ^^^\ versus LPS + NC $p < 0.05$, $p < 0.01$.

contributing to pyroptosis and M1 polarization during the development of liver fibrosis.²⁶ Our results indicated that overexpression of USP8 attenuated lipopolysaccharide-induced upregulation of NLRP3, GSDMD-N, caspase-1, IL-1 β , and IL-18 in BEAS-2B to suppress pyroptosis. NF- κ B signaling also regulated GSDMD-mediated pyroptosis,²⁷ and inhibition of NF- κ B signaling repressed NLRP3 inflammasome-dependent pyroptosis.²⁸ Therefore, USP8 might have promoted inactivation of NF- κ B signaling to suppress pyroptosis in asthma.

PI3K is a promising target for asthma,²⁹ and inhibition of PI3K/AKT promoted airway inflammation in asthma.³⁰ In addition, inhibition of USP8 reduced the expression of p-AKT and p-PI3K to suppress tumor cell proliferation and metastasis.^{31,32} Activation of PI3K/AKT signaling inhibited Foxo1-mediated GSDMD expression, thus alleviating pyroptosis.³³ Our results showed that overexpression of USP8 attenuated lipopolysaccharide-induced decrease of PI3K and AKT phosphorylation in BEAS-2B, suggesting that USP8 promoted activation of PI3K/AKT signaling to suppress pyroptosis and airway inflammation in asthma.

In summary, USP8 exerted an anti-inflammatory effect and suppressed pyroptosis in lipopolysaccharide-treated BEAS-2B by activating PI3K/AKT signaling and inactivating NF- κ B signaling. USP8 might be a potential target for the prevention of asthma. However, the role of USP in the animal model of asthma should be investigated in further research.

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Not applicable.

Competing Interests

The authors state that there are no conflicts of interest to disclose.

Ethics Approval

Not applicable.

Statement of Human and Animal Rights

Not applicable.

Statement of Informed Consent

Not applicable.

Contribution of Authors

Lu Liu and Liting Huan designed the study and carried them out; Yu Zhang, Wei Wei, and Zhihai Chen supervised data

collection; Di Xu analyzed the data; Xiufeng Huang interpreted the data; and Yaoxi Tan, Hongxing Li, and Lu Liu prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

References

- Hallit S, Sacre H, Kheir N, Hallit R, Waked M, Salameh P. Prevalence of asthma, its correlates, and validation of the Pre-School Asthma Risk Factors Scale (PS-ARFS) among preschool children in Lebanon. *Allergol Immunopathol*. 2021;49(1):40-49. <https://doi.org/10.15586/aei.v49i1.25>
- Bagnasco D, Paggiaro P, Latorre M, Folli C, Testino E, Bassi A, et al. Severe asthma: one disease and multiple definitions. *World Allergy Organ J*. 2021;14(11):100606. <https://doi.org/10.1016/j.waojou.2021.100606>
- Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. *Front Pediatr*. 2019;7:246. <https://doi.org/10.3389/fped.2019.00246>
- Iheanacho I, Zhang S, King D, Rizzo M, Ismaila AS. Economic burden of chronic obstructive pulmonary disease (COPD): a systematic literature review. *Int J Chron Obstruct Pulmon Dis*. 2020;15:439-460. <https://doi.org/10.2147/COPD.S234942>
- Wu P, Hartert TV. Evidence for a causal relationship between respiratory syncytial virus infection and asthma. *Expert Rev Anti Infect Ther*. 2011;9(9):731-745. <https://doi.org/10.1586/eri.11.92>
- Sun J, Huang N, Ma W, Zhou H, Lai K. Protective effects of metformin on lipopolysaccharide-induced airway epithelial cell injury via NF- κ B signaling inhibition. *Mol Med Rep*. 2019;19(3):1817-1823. <https://doi.org/10.3892/mmr.2019.9807>
- Wang J. Casticin alleviates lipopolysaccharide-induced inflammatory responses and expression of mucus and extracellular matrix in human airway epithelial cells through Nrf2/Keap1 and NF- κ B pathways. *Phytother Res*. 2018;32(7):1346-1353. <https://doi.org/10.1002/ptr.6067>
- Dufner A, Knobeloch K-P. Ubiquitin-specific protease 8 (USP8/UBPy): a prototypic multidomain deubiquitinating enzyme with pleiotropic functions. *Biochem Soc Trans*. 2019;47(6):1867-1879. <https://doi.org/10.1042/BST20190527>
- Ahmed A, Bowen A, Feng CX, Muhajarine N. Trajectories of maternal depressive and anxiety symptoms from pregnancy to five years postpartum and their prenatal predictors. *BMC Pregn Childbirth*. 2019;19(1):1-10. <https://doi.org/10.1186/s12884-019-2177-y>
- Zhang Y, Luo Y, Wang Y, Liu H, Yang Y, Wang Q. Effect of deubiquitinase USP8 on hypoxia/reoxygenation-induced inflammation by deubiquitination of TAK1 in renal tubular epithelial cells. *Int J Mol Med*. 2018;42(6):3467-3476. <https://doi.org/10.3892/ijmm.2018.3881>
- Zhao J, Bi W, Zhang J, Xiao S, Zhou R, Tsang CK, et al. USP8 protects against lipopolysaccharide-induced cognitive and motor deficits by modulating microglia phenotypes through TLR4/MyD88/NF- κ B signaling pathway in mice. *Brain Behav Immunity*. 2020;88:582-596. <https://doi.org/10.1016/j.bbi.2020.04.052>
- Bigler J, Boedigheimer M, Schofield JPR, Skipp PJ, Corfield J, Rowe A, et al. A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts. *Am J Respir Crit Care Med*. 2017;195(10):1311-1320. <https://doi.org/10.1164/rccm.201604-0866OC>
- Edwards MR, Bartlett NW, Clarke D, Birrell M, Belvisi M, Johnston SL. Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. *Pharmacol Ther*. 2009;121(1):1-13. <https://doi.org/10.1016/j.pharmthera.2008.09.003>

14. de Oliveira JR, da Silva PR, de Paula Rogério A. AT-RvD1 modulates the activation of bronchial epithelial cells induced by lipopolysaccharide and *Dermatophagoides pteronyssinus*. *Eur J Pharmacol*. 2017;805:46-50. <https://doi.org/10.1016/j.ejphar.2017.03.029>
15. Shang J, Liu W, Yin C, Chu H, Zhang M. Cucurbitacin E ameliorates lipopolysaccharide-evoked injury, inflammation and MUC5AC expression in bronchial epithelial cells by restraining the HMGB1-TLR4-NF- κ B signaling. *Mol Immunol*. 2019;114:571-577. <https://doi.org/10.1016/j.molimm.2019.09.008>
16. Qian F-H, Deng X, Zhuang Q-X, Wei B, Zheng D-D. miR-625-5p suppresses inflammatory responses by targeting AKT2 in human bronchial epithelial cells. *Mol Med Rep*. 2019;19(3):1951-1957. <https://doi.org/10.3892/mmr.2019.9817>
17. Weathington NM, Sznajder JI, Mallampalli RK. The emerging role of the ubiquitin proteasome in pulmonary biology and disease. *Am J Respir Crit Care Med*. 2013;188(5):530-537. <https://doi.org/10.1164/rccm.201304-0754PP>
18. El-Hashim A, Renno W, Abduo HT, Jaffal S, Akhtar S, Benter IF. Effect of inhibition of the ubiquitin-proteasome-system and I κ B kinase on airway inflammation and hyperresponsiveness in a murine model of asthma. *Int J Immunopathol Pharmacol*. 2011;24:33-42. <https://doi.org/10.1177/039463201102400105>
19. Chen S, Yun F, Yao Y, Cao M, Zhang Y, Wang J, et al. USP38 critically promotes asthmatic pathogenesis by stabilizing JunB protein. *J Exp Med*. 2018;215(11):2850-2867. <https://doi.org/10.1084/jem.20172026>
20. Bush A. Pathophysiological mechanisms of asthma. *Front Pediatr*. 2019;7:68. <https://doi.org/10.3389/fped.2019.00068>
21. Qiu C, Zhang J, Su M, Fan X. Nuclear factor- κ B mediates the phenotype switching of airway smooth muscle cells in a murine asthma model. *Int J Clin Exp Pathol*. 2015;8(10):12115-12128.
22. Li Z, Zheng J, Zhang N, Li C. Berberine improves airway inflammation and inhibits NF- κ B signaling pathway in an ovalbumin-induced rat model of asthma. *J Asthma*. 2016;53(10):999-1005. <https://doi.org/10.1080/02770903.2016.1180530>
23. Tan Y, Chen Q, Li X, Zeng Z, Xiong W, Li G, et al. Pyroptosis: a new paradigm of cell death for fighting against cancer. *J Exp Clin Cancer Res*. 2021;40(1):153. <https://doi.org/10.1186/s13046-021-01959-x>
24. Zhuang J, Cui H, Zhuang L, Zhai Z, Yang F, Luo G, et al. Bronchial epithelial pyroptosis promotes airway inflammation in a murine model of toluene diisocyanate-induced asthma. *Biomed Pharmacother*. 2020;125:109925. <https://doi.org/10.1016/j.biopha.2020.109925>
25. Wang J, Yuan X, Ding N. IGF2BP2 knockdown inhibits LPS-induced pyroptosis in BEAS-2B cells by targeting caspase 4, a crucial molecule of the non-canonical pyroptosis pathway. *Exp Ther Med*. 2021;21(6):593. <https://doi.org/10.3892/etm.2021.10025>
26. Shu B, Zhou Y-X, Li H, Zhang R-Z, He C, Yang X. The METTL3/MALAT1/PTBP1/USP8/TAK1 axis promotes pyroptosis and M1 polarization of macrophages and contributes to liver fibrosis. *Cell Death Discovery*. 2021;7(1):368. <https://doi.org/10.1038/s41420-021-00756-x>
27. Wang Y, Zhu X, Yuan S, Wen S, Liu X, Wang C, et al. TLR4/NF- κ B signaling induces GSDMD-related pyroptosis in tubular cells in diabetic kidney disease. *Front Endocrinol (Lausanne)*. 2019;10:603. <https://doi.org/10.3389/fendo.2019.00603>
28. Peng D, Li J, Deng Y, Zhu X, Zhao L, Zhang Y, et al. Sodium para-aminosalicylic acid inhibits manganese-induced NLRP3 inflammasome-dependent pyroptosis by inhibiting NF- κ B pathway activation and oxidative stress. *J Neuroinflammation*. 2020;17(1):343. <https://doi.org/10.1186/s12974-020-02018-6>
29. Yoo EJ, Ojiaku CA, Sunder K, Panettieri RA, Jr. Phosphoinositide 3-kinase in asthma: novel roles and therapeutic approaches. *Am J Respir Cell Mol Biol*. 2017;56(6):700-707. <https://doi.org/10.1165/rcmb.2016-0308TR>
30. Zou W, Ding F, Niu C, Fu Z, Liu S. Brg1 aggravates airway inflammation in asthma via inhibition of the PI3K/Akt/mTOR pathway. *Biochem Biophys Res Commun*. 2018;503(4):3212-3218. <https://doi.org/10.1016/j.bbrc.2018.08.127>
31. Sun J, Shen D, Zheng Y, Ren H, Liu H, Chen X, et al. USP8 inhibitor suppresses HER-2 positive gastric cancer cell proliferation and metastasis via the PI3K/AKT signaling pathway. *Onco Targets Ther*. 2020;13:9941-9952. <https://doi.org/10.2147/OTT.S271496>
32. Rong Z, Zhu Z, Cai S, Zhang B. Knockdown of USP8 inhibits the growth of lung cancer cells. *Cancer Manag Res*. 2020;12:12415-12422. <https://doi.org/10.2147/CMAR.S259191>
33. Xu S, Wang J, Zhong J, Shao M, Jiang J, Song J, et al. CD73 alleviates GSDMD-mediated microglia pyroptosis in spinal cord injury through PI3K/AKT/Foxo1 signaling. *Clin Transl Med*. 2021;11(1):e269. <https://doi.org/10.1002/ctm2.269>