



## ORIGINAL ARTICLE

## OPEN ACCESS



## TRIM27 suppresses inflammation injuries in pediatric pneumonia by targeting TLR4/NF- $\kappa$ B signaling pathway

Shan Wang<sup>a</sup>, Baoxia Lu<sup>a</sup>, Jia Liu<sup>a</sup>, Yanhong Gu<sup>b\*</sup>

<sup>a</sup>Department of Pediatric Ward Six, Lu'an People's Hospital, Luan Hospital Affiliated of Anhui Medical University, Lu'an, Anhui Province, China

<sup>b</sup>Department of Pediatric, The People's Hospital of Yubei District of Chongqing City, Chongqing, China

Received 9 December 2021; Accepted 28 December 2021

Available online 1 March 2022

### KEYWORDS

tri-domain protein 27 (TRIM27);  
TLR4/NF- $\kappa$ B pathway;  
pediatric pneumonia

### Abstract

**Background:** Pneumonia widely occurs in children and has high global morbidity and mortality. There is an urgent requirement to clarify the underlying mechanism of pediatric pneumonia and definite its potential therapeutic targets. Tri-domain protein 27 (TRIM27) is one of the TRIM protein family members which widely participated in multiple cellular processes.

**Objective:** To assess whether TRIM27 protects against pediatric pneumonia.

**Methods:** A lipopolysaccharide (LPS)-induced inflammation injury model was constructed. The level of TRIM27 in LPS-induced cells was examined. The effects of TRIM27 in cell apoptosis and inflammatory response was evaluated. Moreover, the involvement of TLR4/NF- $\kappa$ B pathway were detected by Immunoblot.

**Results:** We established a lipopolysaccharide (LPS)-induced inflammation injury model. Our data confirmed that LPS-treated WI-38 cells demonstrated a down-regulated expression of TRIM27. Overexpression of TRIM27 effectively reduced apoptosis and up-regulated the inflammatory factors in LPS-treated WI-38 cells. Toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF- $\kappa$ B) pathway acted as a key point in LPS-mediated inflammation injuries, and overexpression of TRIM27 remarkably inhibited the activity of TLR4/NF- $\kappa$ B pathway, indicating the anti-inflammatory effect of TRIM27.

**Conclusion:** In conclusion, TRIM27 protects WI-38 cells against LPS-induced inflammation injuries by inhibiting TLR4/NF- $\kappa$ B pathway.

© 2022 Codon Publications. Published by Codon Publications.

\*Corresponding author: Yanhong Gu, Department of Pediatric, Yubei District People's Hospital of Chongqing, No. 23 Gongyuan North Road, Yubei District, Chongqing 401120, China. Email address: [guyanhong11209@163.com](mailto:guyanhong11209@163.com)

<https://doi.org/10.15586/aei.v50i2.558>

Copyright: Wang S, et al.

License: This open access article is licensed under Creative Commons Attribution 4.0 International (CC BY 4.0). <http://creativecommons.org/>

## Introduction

Pneumonia spreads widely among children and has high morbidity and mortality worldwide, which causes approximately 1.4-million deaths in children annually<sup>1</sup> The high incidence and recurrence of pediatric pneumonia result in worse complications and poor prognosis, and affect growth of children, especially in developing countries<sup>2</sup> Pediatric pneumonia is a serious disease caused by infectious insults and characterized by combinations of local and systemic complications<sup>3</sup> Hence, it is urgent to explore the underlying mechanism of pediatric pneumonia and its potential therapeutic targets.

It is well recognized that the innate immune system acts as a key point in defense against invading pathogens, and the disorder of inflammatory response is an important pathogenesis of pediatric pneumonia. Toll-like receptor 4 (TLR4), a transmembrane protein, can monitor exogenous pathogens through interacting with lipopolysaccharide (LPS), which is the primary part of outer membrane of Gram-negative bacteria, and trigger inflammation to induce the release of various pro-inflammatory cytokines.<sup>4</sup> Emerging evidences have demonstrated that the activation of TLR4 contributes to the activation of nuclear factor kappa B (NF- $\kappa$ B) pathway in pulmonary inflammatory response<sup>4,5</sup> NF- $\kappa$ B combines with the members of the cytoplasmic I $\kappa$ B inhibitor protein family in inactivated state. When the phosphorylation of I $\kappa$ B is activated with LPS treatment, NF- $\kappa$ B shuttles into nucleus to promote the transcription of cytokine genes such as *Interleukin-1 $\beta$*  (IL-1 $\beta$ ) and IL-6,<sup>6</sup> indicating that TLR4/NF- $\kappa$ B axis is a potential target in the clinical therapy of pediatric pneumonia.

Tri-domain protein 27 (TRIM27) is one of the proteins of the tripartite motif-containing (TRIM) family of proteins. TRIM27 is involved in various cell functions, such as cell proliferation, innate immune response, apoptosis, and transcriptional repression<sup>7-10</sup> Several studies have demonstrated that knockdown of TRIM27 induces cell apoptosis in various cells, such as ovarian cancer cells and hepatocyte L02 cells<sup>11,12</sup> Moreover, TRIM27 also exhibits a negative regulation of NF- $\kappa$ B activation<sup>13</sup> Overexpression of TRIM27 significantly suppresses the activity of TLR4/NF- $\kappa$ B signaling pathway and defends against LPS-induced acute injury in human kidney 2 (HK-2) cells<sup>14</sup> TRIM27 serves as a key regulator in inflammation. For example, TRIM27 promotes inflammatory response in lung cancer cells<sup>15</sup> In addition, TRIM27 protects against cardiac ischemia-reperfusion injury by suppression of inflammation<sup>16</sup> Pneumonia is an inflammation-related disease. However, the role of TRIM27 in this disease is still unclear.

This study aimed to uncover whether TRIM27 participates in pediatric pneumonia, and to evaluate whether TRIM27 inhibits the pulmonary inflammatory response and apoptosis upon LPS through targeting TLR4/NF- $\kappa$ B pathway.

## Materials and methods

### Cell culture and treatment

WI-38 cells were obtained from American Type Culture Collection (ATCC, Manassas, USA) and cultured in

ATCC-formulated Eagle's minimum essential medium, supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% Pen/Strep (P/S) at 37°C and 5% CO<sub>2</sub>. LPS (1, 2, 5, and 10  $\mu$ g/mL, Sigma, USA) was used to induce WI-38 cell injury.

### Cell viability assays

In order to assess cell viability, WI-38 cells were trypsinized, counted, and replated in 96-well plates. After different concentrations of LPS treatment (1, 2, 5, and 10  $\mu$ g/mL), cell viability was evaluated by CellTiter 96 (Promega, USA).

### Flow cytometry

WI-38 cells were seeded into 6-well plates and treated with LPS (5  $\mu$ g/mL). After trypsinized and washed thrice with phosphate-buffered saline solution (PBS), WI-38 cells were treated with propidium iodide (PI) and FITC-Annexin V staining (abcam, UK) at 37°C for 15 min and then analyzed by flow cytometry.

### Western blot

Cell lysates were prepared in radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Hangzhou, China) with protease and phosphatase inhibitors. The protein concentration was measured by a BCA Protein Assay kit (Beyotime, Hangzhou, China). Proteins were separated according to different molecular weights by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes. Membranes were incubated with primary antibodies, including TRIM27 (1:500 dilution, ab277103; abcam, UK), Bax (1:500 dilution, ab32503; abcam), Bcl-2 (1:500 dilution, ab32124; abcam), TLR4 (1:500 dilution, ab22048; abcam), p-p65 (1:500 dilution, ab76302; abcam), p65 (1:500 dilution, ab16502; abcam), I $\kappa$ B $\alpha$  (1:500 dilution, #9242, cell signaling), p-I $\kappa$ B $\alpha$  (1:500 dilution, #2859, Cell Signaling), and anti- $\beta$ -actin (1:1000 dilution, ab8226, abcam), and then treated with secondary antibodies (Beyotime, China), and finally tested by Luminata Crescendo HRP substrate through ChemiDoc XRS System (Bio-Rad, PA). The Image Pro software was used in this assay to calculate the intensity of each blot.

### Real-time polymerase chain reaction (RT-PCR)

Total RNA extraction was conducted by the TRIzol reagent (Life Technologies, Rockville, MD). Quantitative PCR (qPCR) was conducted to evaluate the mRNA levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, and IL-1 $\beta$ . The RT-PCR was performed by using the SYBR Green Master Mix (Applied Biosystems, USA). The 2- $\Delta\Delta$ Ct method was used to quantify the results.

### Enzyme-linked immunosorbent assay (ELISA)

The inflammation factor release in supernatants of WI-38 cells was detected by ELISA. Human TNF- $\alpha$  ELISA kit (ab181421), human IL-6 ELISA kit (ab178013), and human IL-1 $\beta$  ELISA kit (ab214025; Abcam, Cambridge, UK) were

obtained to detect the release of these factors in supernatants of WI-38 cells.

## Statistical analysis

GraphPad Prism 7.0 was used for statistical analysis. One-Way ANOVA was used to perform statistical analyses. Data were presented as mean  $\pm$  standard error of mean (SEM).

## Results

### Overexpression of TRIM27 reduced LPS-induced injury in WI-38 cells

In order to explore whether TRIM27 participates in LPS-induced WI-38 cells, we detected the protein level of TRIM27 and viability of WI-38 cells with LPS treatments at various dosages. As displayed in Figure 1A, the protein level of TRIM27 was obviously reduced with administration of LPS (1, 2, 5, and 10  $\mu\text{g/mL}$ ). Meanwhile, Figure 1B shows that the viability of WI-38 cells was remarkably decreased with administration of LPS (2, 5, and 10  $\mu\text{g/mL}$ ). Based on these results, we used 5  $\mu\text{g/mL}$  LPS for subsequent experiments.

In order to further examine the function of TRIM27 in WI-38 cells, we synthesized plasmid vector to overexpress TRIM27. As shown in Figures 1C and 1D, both protein level of TRIM27 and viability of WI-38 cells were significantly increased in pcDNA3.1-TRIM27-transfected cells.

### Overexpression of TRIM27 inhibited LPS-induced apoptosis in WI-38 cells

The results demonstrated that LPS induced significant increase in cell apoptosis, up-regulation of Bax, and

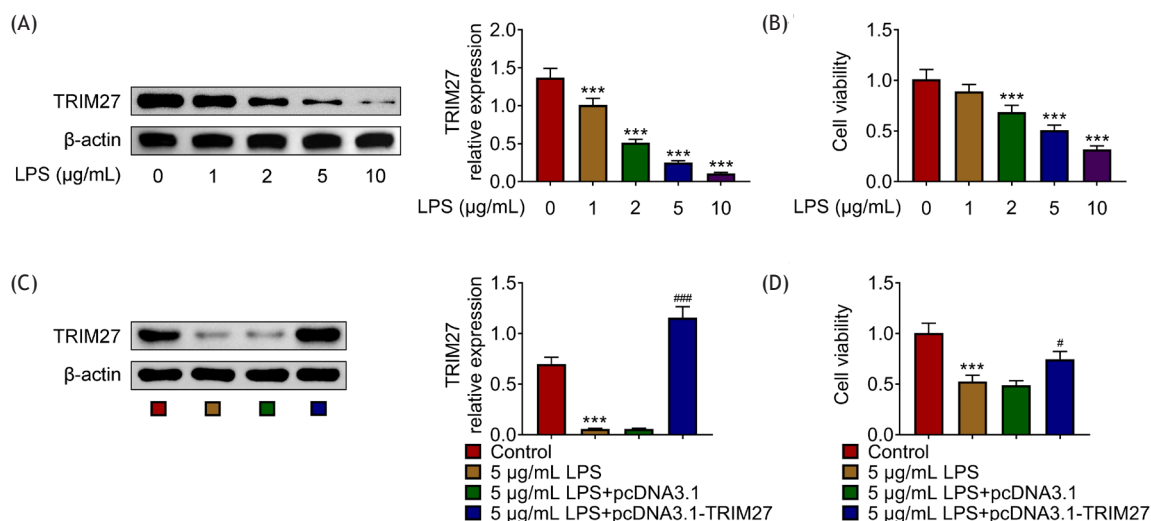
decreased expression of anti-apoptosis marker Bcl-2 in WI-38 cells (Figures 2A-C). Compared with the LPS-treatment group, overexpression of TRIM27 effectively alleviated cell apoptosis and reversed the protein levels of Bax and Bcl-2 in WI-38 cells, indicating that TRIM27 could defend against apoptosis in LPS-induced WI-38 cells.

### Overexpression of TRIM27 decreased LPS-induced inflammatory responses in WI-38 cells

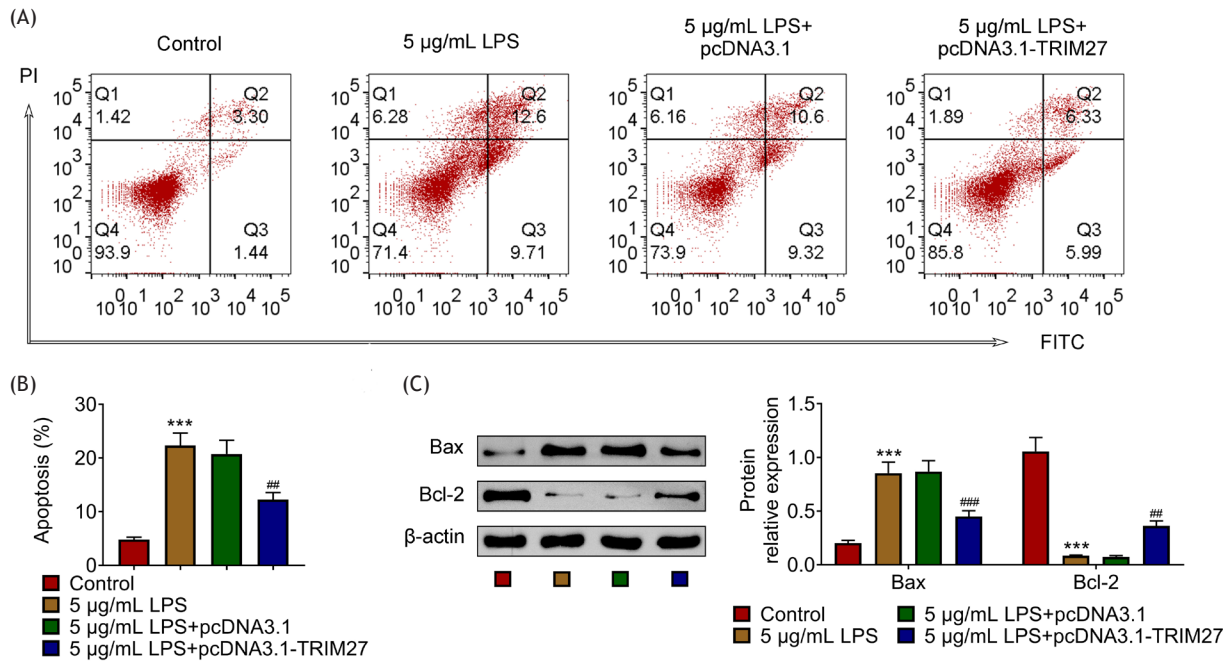
TNF- $\alpha$ , IL-6, and IL-1 $\beta$  are the primary pro-inflammatory cytokines related to inflammatory response. In order to analyze the expression of these factors in LPS-treated WI-38 cells, qRT-PCR and Western blot analysis were used. As exhibited in Figure 3, both mRNA and protein levels of these inflammatory factors were significantly up-regulated in LPS-treated WI-38 cells, while overexpression of TRIM27 attenuated the LPS-induced increase of these factors in WI-38 cells. In addition, through ELISA assays, it was determined that overexpression of TRIM27 could rescue the inflammatory response caused by its depletion, with increased expressions of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  (Supplementary Figure S1).

### Overexpression of TRIM27 inhibited the activity of TLR4/NF- $\kappa$ B signaling pathway

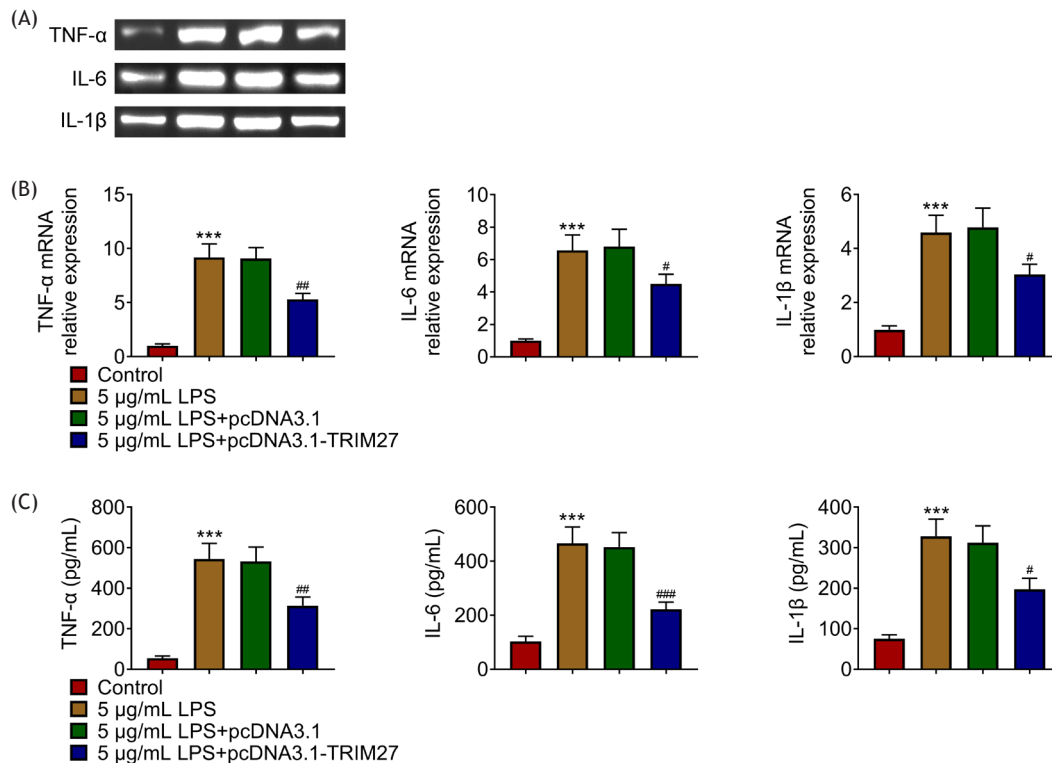
TLR4/NF- $\kappa$ B signaling pathway plays a key role in pneumonia. We determined the protein levels of these key regulators in LPS-treated WI-38 cells. As shown in Figure 4, LPS stimulation resulted in the up-regulation of TLR4 and phosphorylation of p65 and I $\kappa$ B $\alpha$  without affecting the protein levels of p65 and I $\kappa$ B $\alpha$  in WI-38 cells. However, overexpression of TRIM27 decreased the protein levels of TLR4, p-p65, and p-I $\kappa$ B $\alpha$ .



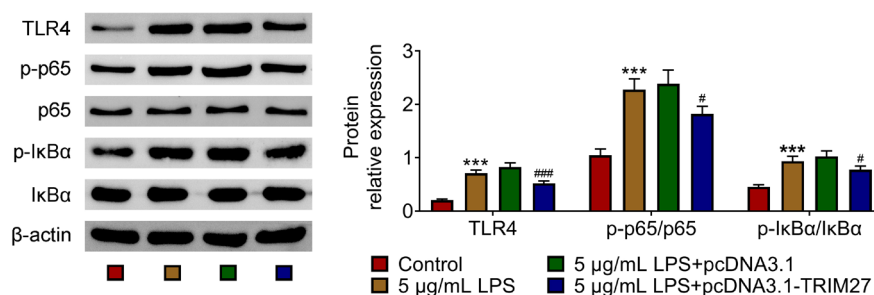
**Figure 1** Overexpression of TRIM27 promoted cell viability in LPS-treated WI-38 cells. (A) Representative images of Western blot results and optical density for the protein blot of TRIM27. (B) Dose-dependent effects of LPS on cell viability. (C) Representative images of Western blot results and optical density for the protein blot of TRIM27 against  $\beta$ -actin. (D) Effect of overexpression of TRIM27 on cell viability. Data were expressed as mean values  $\pm$  standard error of mean;  $n = 5$  per group. \*\* $P < 0.01$  vs. control, # $P < 0.05$ , ### $P < 0.001$  vs. 5- $\mu\text{g/mL}$  LPS.



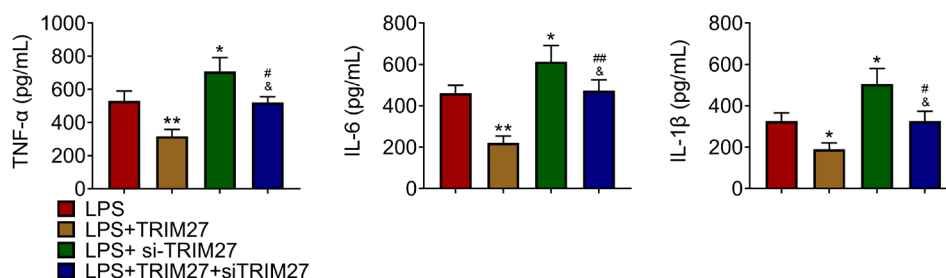
**Figure 2** Overexpression of TRIM27 suppressed cell apoptosis in LPS-treated WI-38 cells. (A) Representative images of flow cytometry for apoptosis assay. (B) Percentage of apoptosis. (C) Representative images of Western blot results and optical density for the protein blot of Bax and Bcl-2 against  $\beta$ -actin. Data were expressed as mean values  $\pm$  standard error of mean;  $n = 5$  per group. \*\* $P < 0.01$  vs. control, ## $P < 0.01$ , ### $P < 0.001$  vs. 5- $\mu$ g/mL LPS.



**Figure 3** Overexpression of TRIM27 suppressed inflammation in LPS-treated WI-38 cells. (A) The gel electrophoresis of PCR products. (B) Effect of overexpression of TRIM27 on the mRNA levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . (C) Effect of overexpression of TRIM27 on the protein levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . Data were expressed as mean values  $\pm$  standard error of mean;  $n = 5$  per group. \*\* $P < 0.01$  vs. control, # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$  vs. 5- $\mu$ g/mL LPS.



**Figure 4** Overexpression of TRIM27 inhibited the activity of TLR4/NF-κB pathway in LPS-treated WI-38 cells. Left: Representative images of Western blot results. Right: Optical density for the protein blot of TLR4 against β-actin, p-p65 against p65 and p-IκBα/IκBα. Data were expressed as mean values ± standard error of mean; n = 5 per group. \*\*\*P < 0.01 vs. control, ###P < 0.001 vs. 5-μg/mL LPS.



**Figure S1** Overexpression of TRIM27 rescued inflammation in LPS-treated WI-38 cells. Effect of overexpression of TRIM27 on protein levels of TNF-α, IL-6, and IL-1β confirmed by ELISA assays. Data were expressed as mean values ± standard error of mean; n = 5 per group. \*P < 0.05, \*\*P < 0.01 vs. control, #P < 0.05.

## Discussion

In this study, the effects of TRIM27 on pediatric pneumonia and the underlying mechanisms were explored through using the model of LPS-induced WI-38 cells, which is widely used in pneumonia studies. Our results demonstrated that apoptosis ratio and inflammation level were prominently increased in cells after administration of LPS, accompanied by the decrease in TRIM27 and Bcl-2 protein levels, as well as cell viability. According to present authors' knowledge, this is the first research to reveal that TRIM27 is a novel therapeutic target in pediatric pneumonia which attenuated LPS-treated WI-38 cells apoptosis and inflammatory response through TLR4/NF-κB signaling pathway. Pediatric pneumonia is a localized inflammatory/infectious event caused by a large variety of microbes in children<sup>17</sup> Emerging evidence indicates that accumulation of cytokine and inflammation plays a key role in the development of pneumonia<sup>18</sup>

TRIM27 has been demonstrated to participate in regulating cell inflammation and apoptosis. The up-regulation of TRIM27 resulted in cell malignancy, while deficiency of TRIM27 attenuated dextran sodium sulfate (DSS)-induced colitis as well as azoxymethane (AOM)/DSS-induced colitis-associated carcinoma (CAC) by impairing

signal transducer and activator of transcription 3 (STAT3) activation<sup>19</sup> In a clinical study, TRIM27 was significantly down-regulated in the liver tissue of liver transplantation patients. In another study, activation of TRIM27 prevented hepatic ischemia-reperfusion injury in mice and hypoxia/reoxygenation treatment in hepatocyte L02 cells<sup>11</sup> However, TRIM27 accelerated cell apoptosis and release of IL-1β through ubiquitination and degradation of peroxisome proliferator-activated receptor gamma (PPARγ) in glutamate-induced neurotoxicity in HT-22 cells, which opposed the previous findings<sup>20</sup> Our results demonstrated that TRIM27 was significantly decreased in LPS-treated WI-38 cells, but the overexpression of TRIM27 effectively increased cell viability.

TLR4 is a receptor of LPS and plays an essential role in pathogen recognition, immunity activation, and inflammation in acute or chronic lung injury mouse model<sup>21</sup> TLR4 could activate NF-κB pathway via MyD88-independent pathway to promote the activation of various inflammatory factors and regulate oxidative stress in the lungs<sup>22,23</sup> NF-κB is the primary nuclear transcription factor. It exists as a homodimer or heterodimer with p50 and p65 proteins bound to the inhibitor IκBα in the cytoplasm of inactive state. Under LPS stimulation, expressions of phosphorylated IκBα and nuclear protein p65 were obviously expedited; meanwhile, activated NF-κB entered the nucleus



and promoted the activation of inflammatory cytokines<sup>24</sup>. Thus, inhibition of TLR4/NF- $\kappa$ B pathway through pharmacological and genomic intervention contributes to protecting against pulmonary inflammation<sup>18,25,26</sup>. In addition, TRIM27 exerted anti-inflammatory and anti-apoptosis functions in LPS-induced HK-2 cells by inhibiting TLR4/NF- $\kappa$ B pathway<sup>14</sup>. Results of the present study have consistently revealed that overexpression of TRIM27 attenuated the activity of TLR4/NF- $\kappa$ B pathway, and effectively suppressed the release of inflammatory cytokines as well as cell apoptosis, compared with LPS-treated group.

## Conclusion

The present study has demonstrated that TRIM27 exerts suppressive effect on pediatric pneumonia. Overexpression of TRIM27 promotes the survival of LPS-treated WI-38 cells by suppressing the expression of apoptosis mediator Bax and the distribution of inflammatory cytokines, and promoting the expression of anti-apoptotic Bcl-2. It is further revealed that TLR4/NF- $\kappa$ B pathway is the downstream signaling of TRIM27. Therefore, TRIM27 serves as a novel therapeutic target for pediatric pneumonia, and its effects are related to the regulation of TLR4/NF- $\kappa$ B pathway.

## Competing interests

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

## Availability of data and material

All data generated and analyzed during this study are included in this published article.

## Authors' contributions

Shan Wang designed the experiments. Jia Liu and Baoxiao Lu conducted experiments and analyzed and interpreted the data. Yanhong Gu prepared the manuscript with contributions from all co-authors.

## References

- McAllister DA, Liu L, Shi T, Chu Y, Reed C, Burrows J, et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: A systematic analysis. *Lancet Glob Health*. 2019;7(1):e47-57. [https://doi.org/10.1016/S2214-109X\(18\)30408-X](https://doi.org/10.1016/S2214-109X(18)30408-X)
- Shah SN, Bachur RG, Simel DL, Neuman MI. Does this child have pneumonia?: The rational clinical examination systematic review. *JAMA*. 2017;318(5):462-71. <https://doi.org/10.1001/jama.2017.9039>
- de Benedictis FM, Kerem E, Chang AB, Colin AA, Zar HJ, Bush A. Complicated pneumonia in children. *Lancet*. 2020;396(10253):786-98. [https://doi.org/10.1016/S0140-6736\(20\)31550-6](https://doi.org/10.1016/S0140-6736(20)31550-6)
- Cao C, Chai Y, Shou S, Wang J, Huang Y, Ma T. Toll-like receptor 4 deficiency increases resistance in sepsis-induced immune dysfunction. *Int Immunopharmacol*. 2018;54:169-76. <https://doi.org/10.1016/j.intimp.2017.11.006>
- Knobloch J, Chikosi SJ, Yanik S, Rupp J, Jungck D, Koch A. A systemic defect in toll-like receptor 4 signaling increases lipopolysaccharide-induced suppression of IL-2-dependent T-cell proliferation in COPD. *Am J Physiol Lung Cell Mol Physiol*. 2016;310(1):L24-39. <https://doi.org/10.1152/ajplung.00367.2014>
- Park JS, Park MY, Cho YJ, Lee JH, Yoo CG, Lee CT, et al. Anti-inflammatory effect of erdosteine in lipopolysaccharide-stimulated RAW 264.7 cells. *Inflammation*. 2016;39(4):1573-81. <https://doi.org/10.1007/s10753-016-0393-4>
- Ma Y, Wei Z, Bast RC, Jr., Wang Z, Li Y, Gao M, et al. Down-regulation of TRIM27 expression inhibits the proliferation of ovarian cancer cells in vitro and in vivo. *Lab Invest*. 2016;96(1):37-48. <https://doi.org/10.1038/labinvest.2015.132>
- Zheng Q, Hou J, Zhou Y, Yang Y, Xie B, Cao X. Siglec1 suppresses antiviral innate immune response by inducing TBK1 degradation via the ubiquitin ligase TRIM27. *Cell Res*. 2015;25(10):1121-36. <https://doi.org/10.1038/cr.2015.108>
- Zaman MM, Nomura T, Takagi T, Okamura T, Jin W, Shinagawa T, et al. Ubiquitination-deubiquitination by the TRIM27-USP7 complex regulates tumor necrosis factor alpha-induced apoptosis. *Mol Cell Biol*. 2013;33(24):4971-84. <https://doi.org/10.1128/MCB.00465-13>
- Jiang J, Xie C, Liu Y, Shi Q, Chen Y. Up-regulation of miR-383-5p suppresses proliferation and enhances chemosensitivity in ovarian cancer cells by targeting TRIM27. *Biomed Pharmacother*. 2019;109:595-601. <https://doi.org/10.1016/j.biopha.2018.10.148>
- Chen SY, Zhang HP, Li J, Shi JH, Tang HW, Zhang Y, et al. Tripartite motif-containing 27 attenuates liver ischemia/reperfusion injury by suppressing transforming growth factor beta-activated kinase 1 (TAK1) by TAK1 binding protein 2/3 degradation. *Hepatology*. 2021;73(2):738-58. <https://doi.org/10.1002/hep.31295>
- Xing L, Tang X, Wu K, Huang X, Yi Y, Huan J. TRIM27 functions as a novel oncogene in non-triple-negative breast cancer by blocking cellular senescence through p21 ubiquitination. *Mol Ther Nucleic Acids*. 2020;22:910-23. <https://doi.org/10.1016/j.omtn.2020.10.012>
- Zheng F, Xu N, Zhang Y. TRIM27 promotes hepatitis C virus replication by suppressing type I interferon response. *Inflammation*. 2019;42(4):1317-25. <https://doi.org/10.1007/s10753-019-00992-5>
- Li XK, Xu XZ, Cong Q, Zhao F, Yang YY, Li AQ, et al. Tri-domain proteins 27 reduce inflammation and apoptosis in HK-2 cells and protect against acute kidney injury in mice. *Eur Rev Med Pharmacol Sci*. 2020;24(23):12258-66.
- Chen EJ, Chen S, Zhou FL. Mechanism of TRIM27 promoting inflammatory response in lung cancer cells. *Zhonghua Zhong Liu Za Zhi (Chinese J Oncol)*. 2021;43(10):1076-81.
- Li Y, Meng Q, Wang L, Cui Y. TRIM27 protects against cardiac ischemia-reperfusion injury by suppression of apoptosis and inflammation via negatively regulating p53. *Biochem Biophys Res Commun*. 2021;557:127-34. <https://doi.org/10.1016/j.bbrc.2021.03.061>
- Jain S, Williams DJ, Arnold SR, Ampofo K, Bramley AM, Reed C, et al. Community-acquired pneumonia requiring hospitalization among US children. *N Engl J Med*. 2015;372(9):835-45. <https://doi.org/10.1056/NEJMoa1405870>
- Nong W. Long non-coding RNA NEAT1/miR-193a-3p regulates LPS-induced apoptosis and inflammatory injury in WI-38 cells through TLR4/NF- $\kappa$ B signaling. *Am J Transl Res*. 2019;11(9):5944-55.
- Zhang HX, Xu ZS, Lin H, Li M, Xia T, Cui K, et al. TRIM27 mediates STAT3 activation at retromer-positive structures to promote

- colitis and colitis-associated carcinogenesis. *Nat Commun.* 2018;9(1):3441. <https://doi.org/10.1038/s41467-018-05796-z>
20. Hao Y, Bai S, Peng J, Hong R, Ding J, Li Z, et al. TRIM27-mediated ubiquitination of PPAR gamma promotes glutamate-induced cell apoptosis and inflammation. *Exp Cell Res.* 2021;400(1):112437. <https://doi.org/10.1016/j.yexcr.2020.112437>
21. Ye R, Liu Z. ACE2 exhibits protective effects against LPS-induced acute lung injury in mice by inhibiting the LPS-TLR4 pathway. *Exp Mol Pathol.* 2020;113:104350. <https://doi.org/10.1016/j.yexmp.2019.104350>
22. Yan J, Li J, Zhang L, Sun Y, Jiang J, Huang Y, et al. Nrf2 protects against acute lung injury and inflammation by modulating TLR4 and Akt signaling. *Free Radic Biol Med.* 2018;121:78-85. <https://doi.org/10.1016/j.freeradbiomed.2018.04.557>
23. Tang J, Xu L, Zeng Y, Gong F. Effect of gut microbiota on LPS-induced acute lung injury by regulating the TLR4/NF-kB signaling pathway. *Int Immunopharmacol.* 2021;91:107272. <https://doi.org/10.1016/j.intimp.2020.107272>
24. Liu Q, Zhang Y, Liu S, Liu Y, Yang X, Liu G, et al. Cathepsin C promotes microglia M1 polarization and aggravates neuroinflammation via activation of Ca(2+)-dependent PKC/p38MAPK/NF-kappaB pathway. *J Neuroinflamm.* 2019;16(1):10. <https://doi.org/10.1186/s12974-019-1398->
25. Liu JX, Li X, Yan FG, Pan QJ, Yang C, Wu MY, et al. Protective effect of forsythoside B against lipopolysaccharide-induced acute lung injury by attenuating the TLR4/NF-kappaB pathway. *Int Immunopharmacol.* 2019;66:336-46. <https://doi.org/10.1016/j.intimp.2018.11.033>
26. Wu F, Liu Y, Lv X, Miao X, Sun Y, Yu W. Small interference RNA targeting TLR4 gene effectively attenuates pulmonary inflammation in a rat model. *J Biomed Biotechnol.* 2012;2012:406435. <https://doi.org/10.1155/2012/406435>