Lipopolysaccharide enhances DNA-induced IFN-β expression and autophagy by upregulating cGAS expression in A549 cells

RONG WANG[1], WEI WANG[1], AILI LI[1], YONGQIN WANG[2-4], JUNFEI JIN[2-4], ZHAOQUAN HUANG[5] and GUOJIN HUANG[1-3]

[1]Laboratory of Respiratory Disease, Affiliated Hospital of Guilin Medical University; [2]China-USA Lipids in Health and Disease Research Center; [3]Guangxi Key Laboratory of Molecular Medicine in Liver Injury and Repair, Guilin Medical University; [4]Laboratory of Hepatobiliary and Pancreatic Surgery, Affiliated Hospital of Guilin Medical University; [5]Department of Pathology, Guilin Medical University, Guilin, Guangxi 541001, P.R. China

Received September 11, 2018; Accepted July 5, 2019

DOI: 10.3892/etm.2019.8001

Abstract. Cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) is a newly identified cytosolic DNA sensor, but its function in lung epithelial cells is relatively unknown. In the present study, the effects of lipopolysaccharide (LPS) on the expression and function of cGAS in the A549 lung epithelial cell line was investigated. The cells were treated with LPS at different concentrations (e.g., 100, 200, 400 and 800 ng/ml), and the cGAS expression levels were examined via western blot analysis and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The cells were pretreated with LPS, followed by *E. coli* DNA transfection using Lipofectamine® 3000. After 24 h, interferon (IFN)-β production was measured using ELISA and the expression of the autophagic markers, microtubule-associated proteins 1A/1B light chain 3 and sequestosome-1, were determined using western blot analysis. The cells were also pretreated with either a toll-like receptor (TLR) 4 inhibitor, a serine/threonine-protein kinase TBK1 (TBK1) inhibitor or an nuclear factor (NF)-κB inhibitor, followed by LPS treatment, and the cGAS expression levels were examined via western blot analysis and RT-qPCR. The result showed that LPS treatment upregulated cGAS expression in a dose-dependent manner. *E. coli* DNA treatment could induce IFN-β production and autophagy via cGAS, which was enhanced by LPS pretreatment. The effect of LPS on cGAS expression was suppressed by treatment with a TLR4 inhibitor, a TBK1 inhibitor and an NF-κB inhibitor. In conclusion, LPS enhances DNA-induced IFN-β production and autophagy by upregulating cGAS expression through the myeloid differentiation primary response protein MyD88-independent TLR4 signaling pathway in A549 cells.

Correspondence to: Dr Guojin Huang, Laboratory of Respiratory Disease, Affiliated Hospital of Guilin Medical University, 15 Lequn Road, Guilin, Guangxi 541001, P.R. China
E-mail: hgjj@163.com

Dr Zhaoquan Huang, Department of Pathology, Guilin Medical University, 15 Lequn Road, Guilin, Guangxi 541001, P.R. China
E-mail: gxlzzq@163.com

Key words: lipopolysaccharide, cyclic GMP-AMP synthase, interferon-β, autophagy, A549 cells

Introduction

Inflammation plays a key role in the pathogenesis of many lung diseases, such as chronic obstructive pulmonary disease (COPD) (1). Accumulating evidence has demonstrated that lung epithelial cells not only form the epithelium, which acts as a physical barrier between the inhaled air and internal tissues, but also are actively involved in antimicrobial defense through pattern recognition receptors (PRRs) on the cell surface and in the cytoplasm (2-4). Cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) is a PRR that can sense cytoplasmic DNA and synthesize cyclic GMP-AMP (cGAMP) (5), which activates the stimulator of interferon genes/serine/threonine-protein kinase 1 TBK1 (TBK1)/interferon regulatory factor (IRF) 3 axis and eventually triggers the expression of type I interferons (IFN-I) (5). Therefore, cGAS has crucial functions in DNA-induced innate immune responses in immune cells (6). As cGAS binds to cytosolic DNA irrespective of DNA sequence, it can be activated by DNA from invading pathogens or from the cell itself (7). In macrophages, cGAS can directly interact with the autophagy protein Beclin-1 to mediate DNA-induced autophagy (8). However, it remains unknown whether cGAS plays a role in regulating autophagy in lung epithelial cells.

Toll-like receptor 4 (TLR4) is a type of PRR that has crucial functions in the pulmonary immune response against bacterial infection (9). TLR4 can recognize and be activated by LPS, an important component of the outer membrane of Gram-negative bacteria (10). On the one hand, TLR4 activation stimulates the production of proinflammatory cytokines through the myeloid differentiation primary response gene 88 (MyD88)-dependent pathway, in which MyD88, interleukin-1 receptor-associated kinase 1, TNF receptor-associated factor (TRAF) 6, and

the transcription factors nuclear factor (NF)-κB, activator protein 1 (AP-1) and IRF-5 are key molecules (11). On the other hand, TLR4 activation also induces the production of IFN-I, such as IFN-β through the MyD88-independent pathways, including Toll-interleukin-1 receptor domain-containing adaptor-inducing interferon-β (TRIF), TRAF3, receptor-interacting serine/threonine-protein kinase 1 (RIP1), TBK1 and the transcription factors, NF-κB, AP-1 and IRF3 (11). LPS can induce autophagy in murine and human macrophages (12), which is regulated through a TRIF-dependent, MyD88-independent TLR4 signaling pathway (12). RIP1 and p38 mitogen-activated protein kinase are downstream components of this pathway (12). LPS also stimulates cGAS expression in an IFN-I-dependent manner in murine and human macrophages (13). However, it remains unknown whether and by what mechanisms LPS stimulates cGAS expression in lung epithelial cells.

Autophagy is a highly conserved cellular pathway by which damaged organelles and proteins are delivered to the lysosome to be degraded by enzymes (14). Autophagy is also involved in the host defense against microbes by degradation of microorganisms and delivery of microbial nucleic acids and antigens for the activation of immune responses (14). Increased levels of autophagy have been observed in lung tissues from patients with COPD (15), and exposure to cigarette smoke extract results in increased autophagy in lung epithelial cells (15). In addition to cigarette smoke, infectious agents also contribute to COPD initiation and progression (16). However, whether and how infectious agents affect autophagy in lung epithelial cells remains unknown.

In the present study, the effects of DNA on IFN-β expression and autophagy and the effects of LPS on the expression and function of cGAS in A549 cells were investigated.

Materials and methods

Antibodies and other reagents. An anti-cGAS (cat. no. 15102; 1:1,000 dilution) antibody was purchased from Cell Signaling Technology, Inc. An anti-β-actin antibody (cat. no. TA-09; 1:1,000 dilution) was obtained from OriGene Technologies, Inc. microtubule-associated proteins 1A/1B light chain 3B (LC3B; cat. no. NB600-1384; 1:1,000 dilution) and sequestosome-1 (p62) antibodies (cat. no. NBP1-48320; 1:1,000 dilution) were obtained from Novus Biologicals LLC. LPS [*Escherichia coli (E. coli)* 055:B5] was purchased from Sigma-Aldrich (Merck KGaA). The TLR4 inhibitor TAK242 (cat. no. 13871), the TBK1 inhibitor BX795 (cat. no. 14932) and the NF-κB inhibitor BAY11-7082 (cat. no. 10010266) were obtained from Cayman Chemical Company. The cGAS inhibitor RU.521 (17) (cat. no. AOB37877) was purchased from Aobious Inc. *E. coli* was purchased from the Beijing CWBIO Company. Recombinant human IFN-β was purchased from Multisciences Biotech Co., Ltd.

Cell culture, treatments and transfection. A549 cell line derived from an alveolar cell carcinoma was used as model of alveolar epithelial cells in the current study (18,19). A549 cells were obtained from the Kunming cell bank of the Chinese Academy of Sciences (Kunming, China). Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum purchased from Thermo Fisher Scientific and 1% penicillin/streptomycin at 37˚C in a humidified incubator with 5% CO_2. A549 cells ($2x10^5$ cells/well) were seeded in six-well plates and cultured at 37˚C in a 5% CO_2 incubator overnight, followed by further experimentation. To test the effects of LPS on cGAS expression, LPS at different concentrations (100, 200, 400 and 800 ng/ml) were used to treat A549 cells for 4 h, and then cGAS expression was analyzed. For inhibitor, A549 cells were pretreated with TAK242 (10 μM), BX-795 (10 μM) or BAY11-7082 (20 μM) for 1 h, followed by LPS (400 ng/ml) treatment for 4 h. For transfection experiments, A549 cells ($2x10^5$ cells/well) were seeded in six-well plates overnight, then transfected with *E. coli* DNA (2 μg/ml) using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The cGAS inhibitor RU.521 was added at the indicated concentrations (0.5, 1, 1.5 and 2 μM) to cell culture wells concurrently with the transfection materials. The control group was treated under the same condition but without DNA and RU.521. A total of 24 h post-transfection, the cells and cell culture media were harvested separately for further analysis.

Western blot analysis. Western blot assays were performed as previously described (20). In brief, A549 cells were collected and lysed with lysis buffer (cat. no. R0020; Beijing, Solarbio Science and Technology Co., Ltd.) on ice for 10 min. The supernatant was obtained by centrifugation at 13,500 x g for 20 min at 4˚C, and the protein concentration of the supernatant was measured with a BCA kit (cat. no. P0009; Beyotime Institute of Biotechnology) according to the manufacturer's instructions. A total of 20 μg protein was loaded per lane and separated by 12 or 15% SDS-PAGE and then transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat milk at room temperature for 1-h, followed by incubation with the primary antibody (LC3B, p62, β-actin and cGAS) at 4˚C overnight. The membrane was then incubated with a horseradish peroxidase-conjugated goat anti-rabbit/mouse secondary antibody at room temperature for 1 h. Blots were developed using an ECL kit (cat. no. P0018; Beyotime Institute of Biotechnology). The gray value of the target protein and β-actin were analyzed using Image J software (version 4.0; National Institutes of Health, Bethesda).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RT-qPCR assays were performed as described previously (21). In brief, total RNA from A549 cells was isolated using TRIzol® (cat. no. 15596026; Thermo Fisher Scientific, Inc.). cDNA was amplified with 2 μg of total RNA from each sample using the RT kit (cat. no. KR106; Tiangen Biotech Co., Ltd) according to the manufacturer's protocol. The FQ-RT primer Mix for cDNA consisted of oligo-dT primer and Random primers (8N). Reverse transcription conditions were as follows: 42˚C for 15 min, then 95˚C for 3 min. RT-qPCR was performed using SYBR Green I (cat. no. RR820A; Takara Bio Inc.) and a CFX touch real-time PCR detection system (Bio-Rad Laboratories, Inc.). GAPDH (forward, 5'-CAGGAGGCATTGCTGATGAT-3' and reverse, 5'-AAGGCTGGGGCTCATTT-3') was used as the internal control. Primers for cGAS (forward, 5'-GTACCCAGAACC CTCAAGACA-3' and reverse, 5'-GTCCTGAGGCACTGA

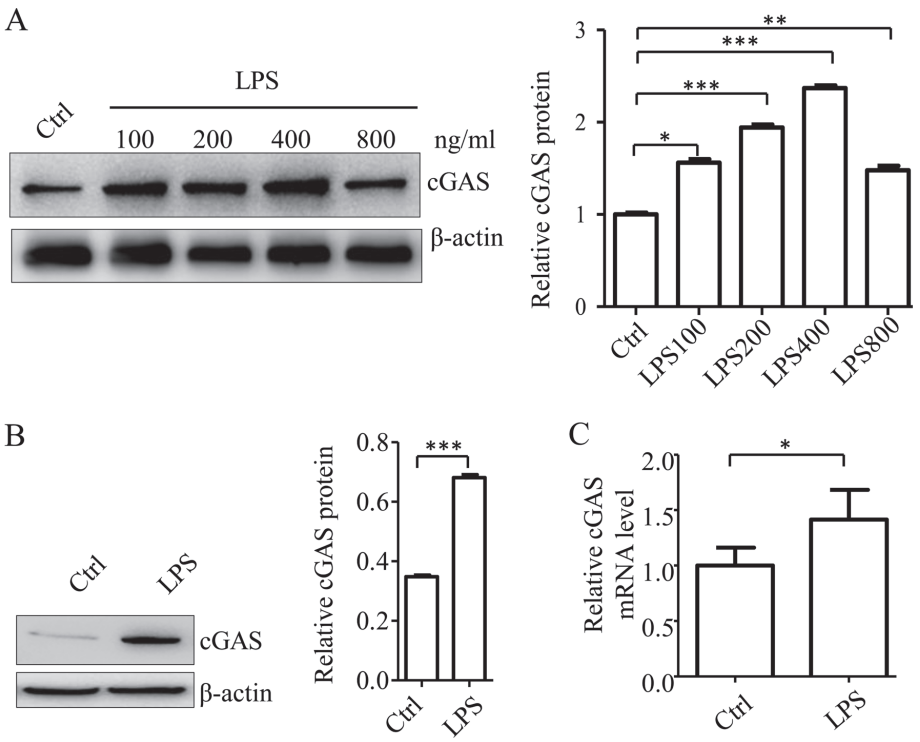

Figure 1. LPS stimulates cGAS expression in A549 cells. (A) LPS stimulates cGAS expression in a dose-dependent manner, between 100 and 400 ng/ml. A549 cells were treated with LPS at the indicated concentrations for 4 h, following which cGAS protein levels were determined using western blot analysis. β-actin was used as the loading control. (B and C) LPS treatment upregulates cGAS expression at the protein and mRNA levels. A549 cells were treated with 400 ng/ml LPS for 4 h, after which the cGAS protein and mRNA levels were determined using (B) western blot analysis and (C) reverse transcription-quantitative polymerase chain reaction. *P<0.05, **P<0.01 and ***P<0.001 with comparisons indicated by lines. Ctrl, control; LPS, lipopolysaccharide; cGAS, cyclic guanosine monophosphate-adenosine monophosphate synthase.

AGAAAG-3') for RT-qPCR were obtained from GeneCopoeia (cat. no. HQP001767). Thermocycling conditions were as follows: 95˚C for 30 sec, 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. The relative mRNA expression level of the genes was determined using the $2^{-\Delta\Delta Cq}$ method (22).

E. coli DNA preparation. An *E. coli* strain (cat. no. CW0808) was purchased from Beijing CWBIO Company and DNA was extracted using a bacterial DNA kit (cat. no. DP302; Tiangen Biotech Co., Ltd) according to the manufacturer's instructions. The DNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.).

ELISA. A549 cells and culture media were collected separately at the end of the various treatments of inhibitors, LPS or *E. coli* DNA alone or combined. Cell lysates were prepared as previously described by adding 200 μl lysis buffer (21). IFN-β levels in the cell culture media and in the supernatant of the cell lysates were measured with a human IFN-β ELISA kit (cat. no. EK1236; Multisciences Biotech Co., Ltd.) according to the manufacturer's instructions. IFN-β levels in the cell culture media and the cell lysates were combined to determine the total IFN-β level under each condition indicated in figures.

Statistical analysis. SPSS v20 (IBM, Corp.) and GraphPad Prism v5 (GraphPad Software Inc.) were used to analyze all data. All experiments were performed independently at least three times, and the results are shown as the mean ± standard error of the mean. Comparison between groups was performed using either the Student's t-test or one-way analysis of variance test followed by the Student-Newman-Keuls post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

LPS treatment upregulates cGAS expression in A549 cells. LPS treatment can upregulate cGAS expression in both murine and human macrophages (13). To assess whether LPS produces the same effects on cGAS expression in lung epithelial cells, A549 cells were treated with LPS at different concentrations (i.e., 100, 200, 400 and 800 ng/ml) for 4 h and cGAS protein levels were then examined. The results showed that LPS treatment induced cGAS expression in A549 cells, and this effect was dose-dependent, from 100-400 ng/ml LPS. The cGAS protein levels in cells treated with 400 ng/ml LPS was higher compared with that in cells treated with 800 ng/ml LPS (Fig. 1A). As 400 ng/ml LPS treatment had the strongest effect on cGAS expression, this concentration was selected for further experiments. To confirm the effect of LPS on cGAS expression at the mRNA level, A549 cells were treated with LPS (400 ng/ml) for 4 h and cGAS mRNA levels were determined using RT-qPCR. The results showed that cGAS mRNA levels in A549 cells were significantly increased compared with the control group, which was consistent with the western blot results (Fig. 1B and C). These findings demonstrated that LPS treatment can induce cGAS expression in A549 cells.

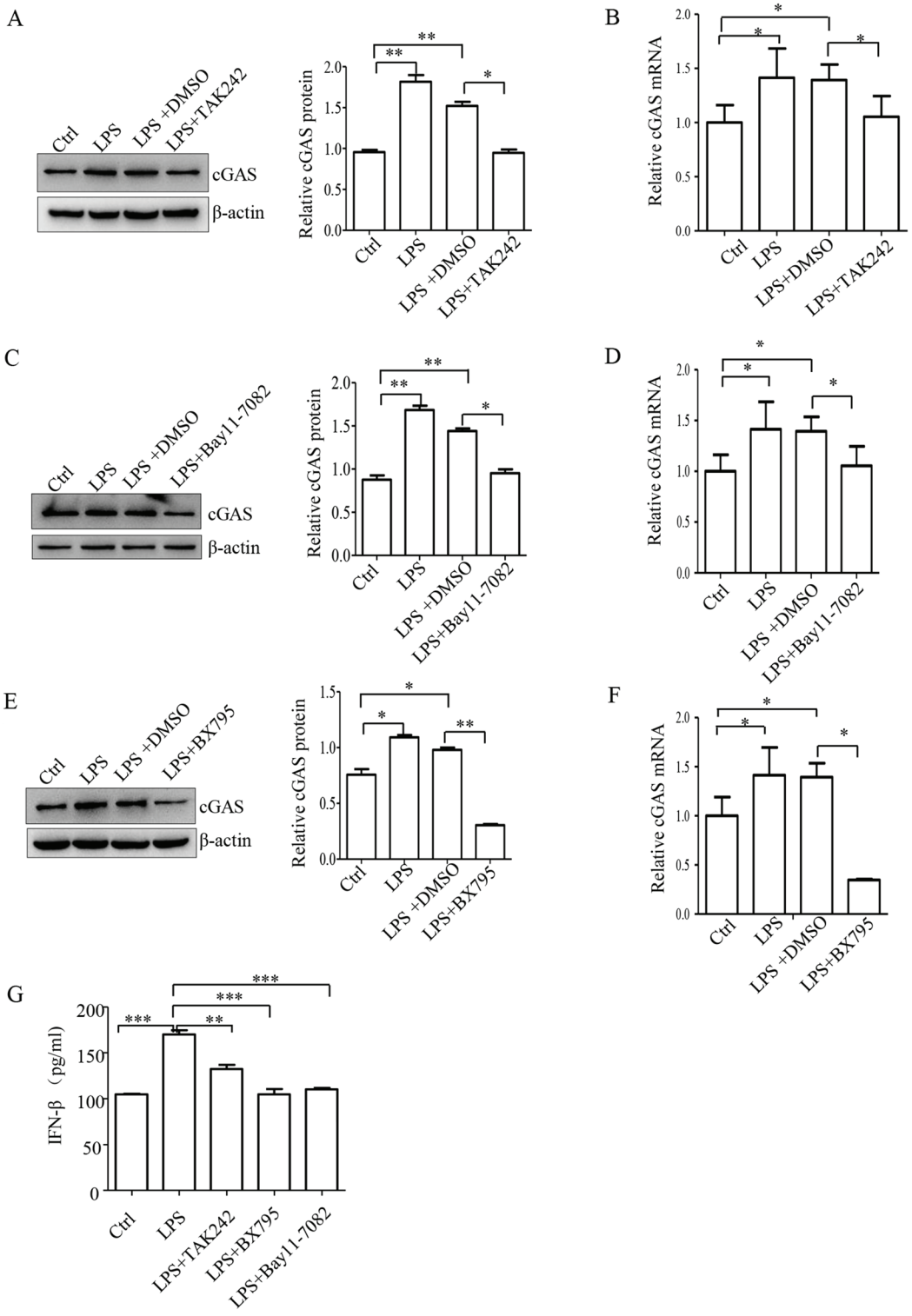

Figure 2. LPS regulates cGAS expression through the MyD88-independent TLR4 signaling pathway in A549 cells. The cells were treated with either (A and B) 10 μM TAK242 TLR4 inhibitor, (C and D) 20 μM Bay11-7082 NF-κB inhibitor or (E and F) 10 μM BX-795 TBK1 inhibitor for 1 h, followed by 400 ng/ml LPS treatment for 4 h. (A, C and E) cGAS protein levels were determined using western blot analysis. β-actin was used as a loading control. (B, D and F) cGAS mRNA levels were determined using reverse transcriptase-quantitative polymerase chain reaction. (G) TAK242, Bay11-7082 and BX-795 treatment inhibit IFN-β expression induced by LPS. The cells were treated with TAK242 (10 μM), BX795 (10 μM), Bay11-7082 (20 μM) for 1 h, respectively, followed with LPS treatment for 4 h. IFN-β expression was measured using ELISA. *P<0.05, **P<0.01 and ***P<0.001 with comparisons indicated by lines. cGAS, cyclic guanosine monophosphate-adenosine monophosphate synthase; Ctrl, control; LPS, lipopolysaccharide; IFN-β, interferon-β.

LPS regulates cGAS expression through the MyD88-independent TLR4 pathway in A549 cells. LPS stimulates cGAS expression in an IFN-I-dependent manner in immune cells (13), and LPS binds to TLR4 and further activates the MyD88-independent pathway to trigger IFN-I production in immune cells (11). TAK242 is a selective TLR4 inhibitor that interferes with the interaction between TLR4 and its adaptor molecules (23). To elucidate the mechanisms by which LPS

upregulates cGAS in A549 cells, the cells were pretreated with TAK242 (10 μM) for 1 h, followed by LPS treatment for 4 h, and subsequently the cGAS protein and mRNA levels were determined. The results showed that TLR4 inhibitor treatment blocked the effect of LPS on cGAS expression at the protein and mRNA levels (Fig. 2A and B), indicating that TLR4 is essential for LPS-stimulated cGAS expression.

BAY 11-7082 is an irreversible inhibitor of IκB, which inhibits cytokine-induced activation of NF-κB (24) and BX795 is a TBK1 inhibitor that can selectively block IRF3, but not NF-κB signaling (25). To further reveal the roles of NF-κB and TBK1, which are two key molecules downstream of TLR4 in the MyD88-independent pathway, on cGAS expression upon LPS treatment, A549 cells were pretreated with either Bay11-7082 or BX795 for 1 h, followed by LPS treatment for 4 h, following which the cGAS protein and mRNA levels were determined. The results showed that treatment with both the NF-κB inhibitor and TBK1 inhibitor significantly reduced LPS-stimulated cGAS expression (Fig. 2C-F), indicating that LPS stimulates cGAS expression through both NF-κB and TBK1 signaling. The efficacy of these inhibitors was validated by exposing cells to these inhibitors for the indicated times, and then assessing the total IFN-β expression levels using ELISA. The results demonstrated that all three inhibitors significantly blocked IFN-β expression induced by LPS (Fig. 2G), indicating they functioned properly. Taken together, these data suggest that LPS induces cGAS expression through the MyD88-independent TLR4 signaling pathway in A549 cells.

LPS enhances DNA-induced IFN-β expression in A549 cells. cGAS can sense cytosolic DNA to promote IFN-I expression in bone marrow-derived macrophage (BMM) cells (13). To determine whether the same function occurs in A549 cells, cells were transfected with *E. coli* DNA, and IFN-β levels in the cell culture media and in cell lysates were measured using ELISA 24 h post-transfection. The results are shown in Fig. 3, which indicates that IFN-β expression in cells transfected with DNA were significantly increased (over 1.5-fold) compared with that in the control cells. As LPS could upregulate cGAS expression in A549 cells, the authors hypothesized that LPS might enhance DNA-induced IFN-β production. To test this hypothesis, A549 cells were pretreated with LPS for 4 h. Then, LPS was removed, and cells were transfected with DNA. The total IFN-β levels were measured using ELISA 24 h post-transfection. The results showed that LPS pretreatment caused >2.5-fold increase in DNA-induced IFN-β in A549 cells compared with that in the untreated control cells, whereas LPS treatment alone caused an ~20% increase in IFN-β compared with untreated control cells (Fig. 3). These findings indicate that LPS pretreatment enhances DNA-induced IFN-β production. To evaluate the role of cGAS in DNA-induced IFN- β production and to assess whether LPS enhances DNA-induced IFN-β expression through cGAS, the cells were pretreated with or without either LPS, followed by DNA transfection and treatment with cGAS inhibitor RU.521 (2 μM) (17). A total of 24 h after transfection, IFN-β production was measured using ELISA. The results demonstrated that RU.521 treatment significantly blocked the IFN-β increase caused by DNA addition compared with the LPS+DNA group and LPS alone group, and completely inhibited the enhancing effect of LPS on DNA-stimulated IFN-β production compared with DNA alone group (Fig. 3), indicating that LPS enhances DNA-induced IFN-β production via cGAS.

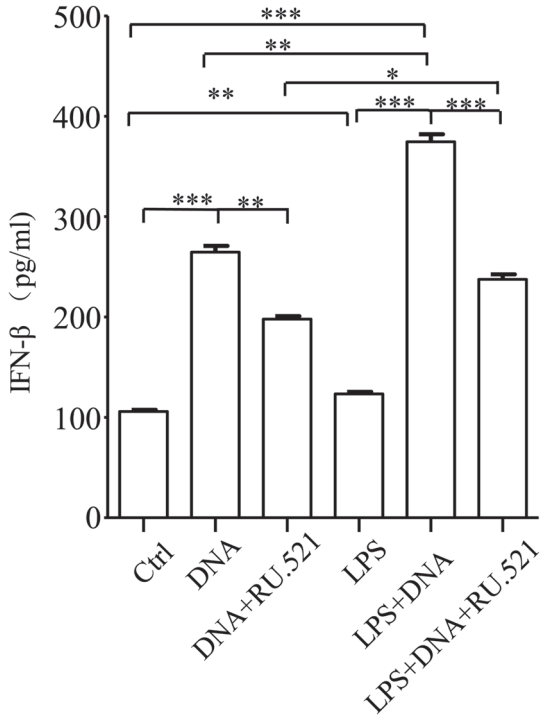

Figure 3. LPS enhances DNA-induced IFN-β expression in A549 cells. A549 cells were pretreated with or without 400 ng/ml LPS for 4 h, followed by 2 μg/ml DNA transfection and treatment with 2 μM RU.521 where indicated. IFN-β levels in culture media and cell lysates were measured using ELISA, 24 h post-transfection. *P<0.05, **P<0.01 and ***P<0.001 with comparisons indicated by lines. Ctrl, control; LPS, lipopolysaccharide; IFN-β, interferon-β.

LPS enhances cGAS-mediated autophagy in A549 cells. It is well-known that LC3B conversion (LC3-I to LC3-II) and lysosomal degradation of LC3-II reflect the progression of autophagy, and that the p62 protein is degraded by autophagy (26). Therefore, LC3B and p62 can both serve as markers of autophagy (26). As a previous study reported that cGAS mediates DNA-induced autophagy in macrophages (8), whether DNA could induce autophagy in A549 cells was investigated in the present study by transfecting with *E. coli* DNA (2 μg/ml). LC3B and p62 levels were measured using western blot analysis, 24 h after transfection. The results showed that DNA treatment led to increased LC3-II expression, along with a decreased p62 expression (Fig. 4A), implying that DNA can induce autophagy in A549 cells. To determine the effect of LPS treatment on DNA-induced autophagy, A549 cells were pretreated with LPS for 4 h and then transfected with *E. coli* DNA (2 μg/ml). LC3B and p62 levels were analyzed using a western blot assay, 24 h following transfection. The results demonstrated that LPS pretreatment significantly promoted LC3-II and p62 degradation caused by the addition of the DNA comparing LPS+DNA group with DNA only group (Fig. 4A), suggesting that LPS enhances the effects of DNA on autophagy in A549 cells. To evaluate the role of cGAS in DNA-induced autophagy, cells were treated with RU.521 at

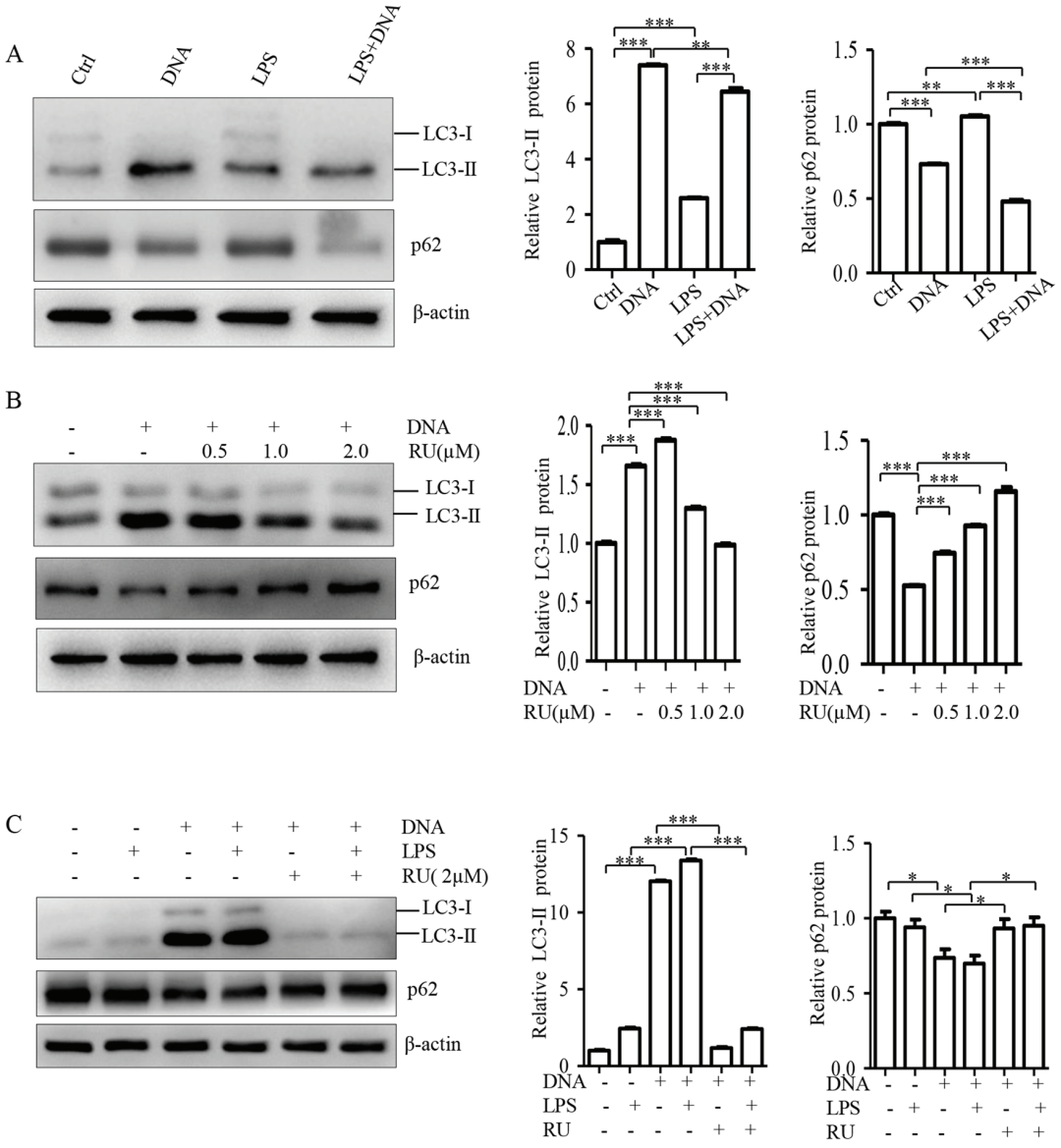

Figure 4. LPS enhances cGAS-mediated autophagy in A549 cells. Cells were either (A) pretreated with or without 400 ng/ml LPS for 4 h, followed by 2 μg/ml DNA transfection, (B) treated with the cGAS inhibitor RU at the indicated concentration and subsequently transfected with 2 μg/ml DNA or (C) pretreated with or without 400 ng/ml LPS for 4 h, followed by 2 μM RU treatment, and transfected with 2 μg/ml DNA. Following this cells were harvested 24-h post-transfection, and the protein expression of LC3-II and p62 was analyzed using western blot analysis. *P<0.05, **P<0.01 and ***P<0.001 with comparisons indicated by lines. RU, RU.521; ctrl, control; LPS, lipopolysaccharide; LC3, microtubule-associated proteins 1A/1B light chain 3B.

0.5, 1.0 and 2.0 μM together with *E. coli* DNA stimulation, and autophagic marker levels were measured using a western blot analysis. The results showed that RU.521 significantly inhibited LC3-I to LC3-II conversion and p62 degradation (Fig. 4B), indicating that DNA-induced autophagy in A549 cells is mediated by cGAS. To evaluate whether the effect of LPS on DNA-induced autophagy is mediated by cGAS, cells were pretreated with LPS, followed by treatment with RU.521 (2.0 μM) and *E. coli* DNA. The autophagy marker levels were examined using western blot analysis. The results showed that RU.521 blocked the effects of LPS on DNA-induced autophagy (Fig. 4C), suggesting that LPS promotes DNA-induced autophagy via cGAS. Although there is difference between LC3-II proteins of DNA+LPS group, the results in Fig. 4A and C both show increased autophagy in DNA+LPS group. Lower LC3-II protein in Fig. 4A was due to an intense autophagic flux that consumes this protein, which is consistent with lower p62 in Fig. 4A.

Discussion

Lung epithelial cells play a key role in immune responses during the pathogenesis of many chronic pulmonary diseases, but the underlying mechanisms of their involvement remain unclear (27). A useful model of type II alveolar epithelial cells are A549 cells, although it is a cancerous cell line derived from an alveolar cell carcinoma (18,19). In the present study, cGAS mediates A549 cellular responses against cytosolic DNA, providing evidence that cGAS are active in A549 cells. Although DNA is absent in the cytoplasm

of lung epithelial cells under normal conditions, it may be released into the cytoplasm to cause immune responses under certain abnormal conditions, such as infection (28). When a patient is infected with bacteria or DNA viruses, the pathogen's genomic DNA may enter the cytoplasm of lung epithelial cells through endocytosis (29,30). Under these circumstances, cGAS can be activated to stimulate the expression of IFN-I, which in turn activates host immune responses through IFN signaling pathway (31). Therefore, the findings from the present study suggest potential mechanisms by which lung epithelial cells react against invading bacteria and DNA viruses. As viral and bacterial infection is a common cause of the exacerbation of COPD (16), these findings may also provide clues to understanding the pathophysiology of COPD.

Enhanced levels of autophagy have been observed in COPD-affected lung tissues (15), suggesting that autophagy may play an important role in COPD. However, how autophagy is regulated in COPD remains unclear. Since cGAS is required for DNA-induced autophagy in BMMs (8), the effects of DNA on autophagy stimulation in lung epithelial cells was evaluated. The results show that DNA transfection can cause autophagy in lung epithelial cells and that cGAS plays a vital role during this process, suggesting a potential mechanism underlying how autophagy is regulated in COPD-affected lung epithelia.

Given that cGAS plays such an important role in lung epithelial cells, how cGAS expression is modulated in these cells was investigated. A previous study reported that in BMMs LPS could stimulate cGAS expression through the MyD88-independent TLR4 signaling pathway (13). Therefore, whether cGAS expression is regulated by the same mechanism in lung epithelial cells was analyzed. In A549 cells, LPS could stimulate cGAS expression in a dose-dependent manner, from 100 to 400 ng/ml. Interestingly, 400 ng/ml LPS treatment had a stronger effect on cGAS expression compared with 800 ng/ml LPS, indicating there might be a negative control mechanism to regulate cGAS expression, which might be triggered by high concentrations of LPS, such as 800 ng/ml. Further experiments are required to address this question in the future. Treatment with the TLR4 inhibitor TAK242, the NF-κB inhibitor Bay11-7082 or the TBK1 inhibitor BX795 could completely block the effects of LPS treatment on cGAS expression, indicating that TLR4, NF-κB and TBK1, which are all key components of the MyD88-independent TLR4 signaling pathway, are required for LPS-induced cGAS expression. Therefore, LPS regulates cGAS expression through the MyD88-independent TLR4 signaling pathway in A549 cells.

In summary, the present study demonstrates that LPS can enhance DNA-induced IFN-β production and autophagy by upregulating cGAS expression through the MyD88-independent TLR4 signaling pathway in A549 cells. This suggests that there is crosstalk between the TLR4 signaling pathway and the cGAS signaling pathway in lung epithelial cells.

Acknowledgements

Not applicable.

Funding

The present study was supported in part by the National Natural Science Foundation of China (grant nos. 81560453 and 81660008), the Natural Science Foundation of Guangxi (grant nos. 2015GXNSFAA139178 and 2015GXNSFBA139168), the Guangxi Health and Family Planning Commission (grant no. S2015-34), and the Lijiang Scholar Award. GH was supported by the Hundred Talents Program of Guangxi.

Availability of data and materials

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

Authors' contributions

RW, WW, AL performed experiments. RW, YW and GH analyzed and interpreted the data. RW, GH, JJ and ZH designed the experiments and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bagdonas E, Raudoniute J, Bruzauskaite I and Aldonyte R: Novel aspects of pathogenesis and regeneration mechanisms in COPD. Int J Chron Obstruct Pulmon Dis 10: 995-1013, 2015.
2. Leiva-Juárez MM, Kolls JK and Evans SE: Lung epithelial cells: Therapeutically inducible effectors of antimicrobial defense. Mucosal Immunol 11: 21-34, 2018.
3. Dudek M, Puttur F, Arnold-Schrauf C, Kühl AA, Holzmann B, Henriques-Normark B, Berod L and Sparwasser T: Lung epithelium and myeloid cells cooperate to clear acute pneumococcal infection. Mucosal Immunol 9: 1288-1302, 2016.
4. Brazee P, Dada LA and Sznajder JI: Role of linear ubiquitination in health and disease. Am J Respir Cell Mol Biol 54: 761-768, 2016.
5. Sun L, Wu J, Du F, Chen X and Chen ZJ: Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339: 786-791, 2013.
6. Li T and Chen ZJ: The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. J Exp Med 215: 1287-1299, 2018.
7. Cai X, Chiu YH and Chen ZJ, The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. Mol Cell 54: 289-296, 2014.
8. Liang Q, Seo GJ, Choi YJ, Kwak MJ, Ge J, Rodgers MA, Shi M, Leslie BJ, Hopfner KP, Ha T, *et al*: Crosstalk between the cGAS DNA sensor and beclin-1 autophagy protein shapes innate antimicrobial immune responses. Cell Host Microbe 15: 228-238, 2014.
9. Sender V and Stamme C: Lung cell-specific modulation of LPS-induced TLR4 receptor and adaptor localization. Commun Integr Biol 7: e29053, 2014.
10. Qiao S, Luo Q, Zhao Y, Zhang XC and Huang Y: Structural basis for lipopolysaccharide insertion in the bacterial outer membrane. Nature 511: 108-111, 2014.

11. Lu YC, Yeh WC and Ohashi PS: LPS/TLR4 signal transduction pathway. Cytokine 42: 145-151, 2008.
12. Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejska KE and Eissa NT: Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. Immunity 27: 135-144, 2007.
13. Ma F, Li B, Liu SY, Iyerz SS, Yu Y, Wu A and Cheng G: Positive feedback regulation of type I IFN production by the IFN-inducible DNA sensor cGAS. J Immunol 194: 1545-1554, 2015.
14. Levine B and Kroemer G: Autophagy in the pathogenesis of disease. Cell 132: 27-42, 2008.
15. Chen ZH, Kim HP, Sciurba FC, Lee SJ, Feghali-Bostwick C, Stolz DB, Dhir R, Landreneau RJ, Schuchert MJ, Yousem SA, *et al*: Egr-1 regulates autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. PLoS One 3: e3316, 2008.
16. Tuder RM and Petrache I: Pathogenesis of chronic obstructive pulmonary disease. J Clin Invest 122: 2749-2755, 2012.
17. Vincent J, Adura C, Gao P, Luz A, Lama L, Asano Y, Okamoto R, Imaeda T, Aida J, Rothamel K, *et al*: Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. Nat Commun 8: 750, 2017.
18. Lieber M, Smith B, Szakal A, Nelson-Rees W and Todaro G: A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int J Cancer 17: 62-70, 1976.
19. Foster KA, Oster CG, Mayer MM, Avery ML and Audus KL: Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. Exp Cell Res 243: 359-366, 1998.
20. Wang Q, Li A, Jin J and Huang G: Targeted interfering DEP domain containing 1 protein induces apoptosis in A549 lung adenocarcinoma cells through the NF-κB signaling pathway. Onco Targets Ther 10: 4443-4454, 2017.
21. Li A, Wang Q, He G, Jin J and Huang G: DEP domain containing 1 suppresses apoptosis via inhibition of A20 expression, which activates the nuclear factor κB signaling pathway in HepG2 cells. Oncol Let 16: 949-955, 2018.
22. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
23. Matsunaga N, Tsuchimori N, Matsumoto T and Ii M: TAK-242 (resatorvid), a small-molecule inhibitor of toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. Mol Pharmacol 79: 34-41, 2011.
24. Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T and Gerritsen ME: Novel inhibitors of cytokine-induced IkappaBalpha hosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. J Biol Chem 272: 21096-21103, 1997.
25. Clark K, Plater L, Peggie M and Cohen P: Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB Kinase epsilon: A distinct upstream kinase mediates Ser-172 phosphorylationand activation. J Biol Chem 284: 14136-14146, 2009.
26. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, Adachi H, Adams CM, Adams PD, Adeli K, *et al*: Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy 12: 1-222, 2016.
27. Biswal S, Thimmulappa RK and Harvey CJ: Experimental therapeutics of Nrf2 as a target for prevention of bacterial exacerbations in COPD. Proc Am Thorac Soc 9: 47-51, 2012.
28. Kawasaki T and Kawai T, Discrimination between self and non-self-nucleic acids by the innate immune system. Int Rev Cell Mol Biol 344: 1-30, 2019.
29. Lu C, Zhang X, Ma C, Xu W, Gan L, Cui J, Yin Y and Wang H: Nontypeable haemophilus influenzae DNA stimulates type I interferon expression via STING signaling pathway. Biochim Biophys Acta Mol Cell Res 1865: 665-673, 2018.
30. Lam E and Falck-Pedersen E: Unabated adenovirus replication following activation of the cGAS/STING-dependent antiviral response in human cells. J Virol 88: 14426-14439, 2014.
31. Ivashkiv LB and Donlin LT: Regulation of type I interferon responses. Nat Rev Immunol 14: 36-49, 2014.