# HIF-1α promotes NLRP3 inflammasome activation in bleomycin-induced acute lung injury

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**Abstract.** The inflammatory response is one of the most important factors in the occurrence and development of acute lung injury (ALI). Hypoxia-inducible factor-1α (HIF-1α) and the NOD-like receptor 3 (NLRP3) inflammasome have been demonstrated to serve an important role in the pathogenesis of ALI. The objective of the present study was to investigate whether HIF-1α could regulate activation of the NLRP3 inflammasome and its potential function and specific mechanism in bleomycin (BLM)-induced ALI. Activation of the NLRP3 inflammasome and secretion of IL-1β were detected following silencing of HIF-1α or NF-κB, respectively, in BLM-treated A549 and RLE-6TN cells. The results demonstrated that the NLRP3 inflammasome could be activated after BLM treatment. HIF-1α and NF-κB expression significantly increased in the BLM group. The levels of NF-κB- and NLRP3 inflammasome-associated proteins, including NLRP3, apoptosis-associated speck-like protein containing CARD and caspase-1, markedly decreased after treating A549 and RLE-6TN cells with HIF-1α small interfering RNA. Activation of the NLRP3 inflammasome was also inhibited after silencing NF-κB. Furthermore, the levels of IL-1β markedly decreased in the cellular culture supernatants following inhibition of HIF-1α and NF-κB. Therefore, the present study indicated that HIF-1α could modulate the activation of the NLRP3 inflammasome and the secretion of IL-1β through NF-κB signaling in BLM-induced ALI. The current results improve understanding of the mechanism of ALI and may provide new ideas for identifying therapeutic targets of ALI.

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*Abbreviations:* ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ASC, apoptosis-associated speck-like protein containing CARD; BLM, bleomycin; HIF-1α, hypoxia-inducible factor-1 α; IL-1β, interleukin-1β; NF-κB, nuclear factor-κB; NLRP3, NOD-like receptor 3; TNF-α, tumor necrosis factor-α

*Key words:* acute lung injury, NOD-like receptor 3 inflammasome, hypoxia-inducible factor-1 α; NF-κB, IL-1β

## Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are caused by multiple factors, which characterize persistent hypoxemia, neutrophil infiltration, disseminated alveolar damage, acute inflammatory syndrome and impaired blood gas barrier (1,2). Numerous studies have focused on the mechanism and therapy of ALI (3-5); however, there are still no effective therapeutic methods in the clinic. In previous years, inflammation has been considered to serve a key role in the development of ALI. However, specific mechanisms have not yet been fully elucidated. Inflammatory factors, including nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, could increase the damage to endothelial and epithelial cells (6,7). Therefore, it is important to further study the mechanism of inflammation in ALI.

The inflammasome is a protein complex that includes the receptor protein apoptosis-associated speck-like protein containing CARD (ASC) and effector molecules, including pre-caspase-1 and pro-caspase-1. The inflammasome can promote the maturation of pro-IL-1β by activating caspase-1. Subsequently, IL-1β is released to the extracellular environment to participate in inflammation, injury and other processes (8,9). Receptor proteins include NOD like receptors (NLRs), including NLRP1 and NLRP3, and the interferon-inducible p200-proteins, including absent in melanoma 2. Different receptor proteins can be activated by different endogenous

or exogenous stimuli. The NLRP3 inflammasome is the most studied inflammasome and has been demonstrated to be activated by numerous factors, including *Listeria*, *Aeromonas*, ATP and insoluble crystals, including uric acid crystal, silica and asbestos (10,11). It has been reported that in ALI, pulmonary fibrosis, chronic obstructive pulmonary disease, asthma and other lung diseases, the NLRP3 inflammasome serves a key role in inflammation (12). Studies have demonstrated that the NLRP3 inflammasome can be activated through the toll-like receptor 4 signaling pathway and participates in inflammatory injury in ventilator-induced lung injury; the ventilator induced lung injury was markedly alleviated after NLRP3-knockout (13,14). However, to the best of the authors' knowledge, the mechanism of NLRP3 inflammasome activation remains unknown in ALI.

Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that is widely expressed in the body. Under normal conditions, HIF-1α can be degraded via the ubiquitin-proteasome pathway. However, during hypoxia the degradation of HIF-1α is inhibited; therefore, it accumulates and is transferred to the nucleus to promote expression of its target genes (15,16). The lung tissue is in a state of persistent hypoxia and the expression of HIF-1α is significantly increased (17-19). It has been demonstrated that HIF-1α participates in inflammation and promotes the expression of inflammatory factors, including TNF-α, IL-6 and IL-1β, in ALI (20,21). In single-stranded RNA viruses-induced inflammatory reactions, HIF-1α has been reported to activate the NLRP3 inflammasome, induce the activation of caspase-1 and then convert inactive pro-IL-1β to IL-1β in human THP-1 myeloid macrophages (22). However, whether HIF-1α can regulate the activation of the NLRP3 inflammasome and the potential function of HIF-1α in ALI remain unknown.

Therefore, the aim of the present study was to investigate whether HIF-1α can regulate the activation of the NLRP3 inflammasome and its potential mechanism in bleomycin (BLM)-induced ALI.

## Materials and methods

*Main reagents*. BLM was purchased from Selleck Chemicals. Anti-HIF-1α (1:500; cat. no. BS3514) primary rabbit monoclonal antibody was obtained from BioWorld Technology, Inc. NF-κB p65 (1:1,000; cat. no. sc-8008), ASC (1:500; cat. no. sc-22514) and caspase-1 (1:500; cat. no. sc-56036) primary mouse monoclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. Anti-NLRP3 (1:1,000; cat. no. ab210491) rabbit monoclonal antibody and anti-β-actin (1:2,000; cat. no. ab179467) primary mouse monoclonal antibody were purchased from Abcam. RIPA buffer, BCA protein assay kit, SDS-PAGE gel preparation kit, 4.0% paraformaldehyde, Triton X-100, DAPI and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1:200; cat. no. A0562) were obtained from Beyotime Institute of Biotechnology.

*Cell culture and transfection*. A549 cells and rat type II alveolar cells (RLE-6TN) were purchased from the American Type Culture Collection. A549 cells were cultured in F-12K medium (Genom Biotech Pvt., Ltd.) with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% antibiotics (100 U/ml penicillin and 100 $\mu$g/ml streptomycin). RLE-6TN cells were grown in DMEM/F-12 (HyClone; GE Healthcare) with 10% FBS and 1% antibiotics (100 U/ml penicillin and 100 $\mu$g/ml streptomycin). Both cell lines were grown at 37˚C in a 5% carbon dioxide incubator. HIF-1α small interfering RNA (siRNA) and NF-κB siRNA were designed and generated by Shanghai Genepharma Co., Ltd. The nucleotides sequences of the HIF-1α, NF-κB p65 (abbreviated as NF-κB) and siControl siRNAs are presented in Table I. Both siRNAs were transfected into A549 and RLE-6TN cells using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. After 1 h, A549 cells were treated with BLM at a concentration of 120 $\mu$M and RLE-6TN cells were treated with BLM at a concentration of 40 $\mu$M for 24 h, according to a previous study (23).

*Western blotting*. Total proteins were extracted from cells with RIPA buffer. Concentrations of the total proteins were determined using a BCA protein assay kit. The total protein samples (4 $\mu$g/$\mu$l) were separated via 10% SDS-PAGE g, transferred to PVDF membranes, blocked with 5% non-fat milk in TBS with 0.1% Tween 20 (TBST) at 37˚C for 1.5 h and incubated with relevant primary antibodies overnight at 4˚C. The secondary antibodies were then incubated with the PVDF membrane for 60 min at room temperature. After the membranes were washed with TBST, the bands were visualized with an ECL detection system (Immobilon Western Chemiluminescent HRP substrate; EMD Millipore), according to the manufacturer's protocol.

*Immunofluorescence*. A549 ($8x10^4$/well) and RLE-6TN ($1x10^5$/well) cells were seeded in confocal dishes 24 h prior to treatment. The cells were the treated with BLM (120 $\mu$M for A549 cells and 40 $\mu$M for RLE-6TN cells) for 24 h. Subsequently, the cells were washed with PBS three times, fixed with 4.0% paraformaldehyde for 10 min at room temperature and permeabilized with 0.5% Triton X-100 for 10 min at room temperature. Subsequently, the cells were incubated with primary antibody (NLRP3, 1:400; Caspase-1, 1:200; ASC, 1:200) overnight at 4˚C and then incubated with FITC-conjugated goat anti-rabbit IgG for 90 min at room temperature. Nuclei were stained with DAPI for 3 min at 37˚C. The cells were observed under a laser confocal microscope (Leica TCS SP8; Leica Microsystems GmbH).

*Reverse transcription-quantitative PCR (RT-qPCR)*. Total RNA was extracted from A549 and RLE-6TN cells with RNAiso Plus reagent (Takara Biotechnology, Co., Ltd.) and the concentration of total RNA in each group was detected with an ultraviolet spectrophotometer. RT (37˚C for 15 min and 85˚C for 5 sec, then stored at 4˚C until further use) was performed using a HiScriptII Q RT SuperMix for qPCR (Vazyme), according to the manufacturer's protocol. qPCR (95˚C for 2 min, followed by 40 cycles of 95˚C for 10 sec, 60˚C for 30 sec and 72˚C for 30 sec) was conducted with a ChamQ™ SYBR qPCR Master mix (Vazyme) using an ABI ViiA™ 7 system. The specific primers for HIF-1α, NF-κB and β-actin were designed and generated by BioTNT. The primer sequences of HIF-1α, NF-κB and β-actin are presented in Table II. All samples were read in triplicate and β-actin served

Table I. The sequences of HIF-1α and NF-κB siRNAs.

| Species | Gene | Sense | Antisense |
|---|---|---|---|
| *Homo sapiens* | HIF-1α | 5'GCCGAGGAAGAACUAUGAATT3' | 5'UUCAUAGUUCUUCCUCGGCTT3' |
| | NF-κB | 5'GGACAUAUGAGACCUUCAA3' | 5'UUGAAGGUCUCAUAUGUCC3' |
| | siControl | 5'UUCUCCGAACGUGUCACGU3' | 5'ACGUGACACGUUCGGAGAA3' |
| *Rattus Norvegicus* | HIF-1α | 5'GGGCCGUUCAAUUUAUGAATT3' | 5'UUCAUAAAUUGAACGGCCCTT3' |
| | NF-κB | 5'AAGAAGCACAGAUACCACCAA3' | 5'UUGGUGGUAUCUGUGCUUCUU3' |
| | siControl | 5'UUCUCCGAACGUGUCACGUTT3' | 5'ACGUGACACGUUCGGAGAATT3' |

HIF-1α, hypoxia-inducible factor-1 α; NF-κB, nuclear factor-κB; siRNA, small interfering RNA.

Table II. The primer sequences of HIF-1α, NF-κB and β-actin used in reverse transcription-quantitative PCR.

| Species | Gene | Primer sequence (Forward primer, 5'-3'; Reverse primer, 5'-3') |
|---|---|---|
| *Homo sapiens* | HIF-1α | GTCTGAGGGGACAGGAGGAT; CTCCTCAGGTGGCTTGTCAG |
| | NF-κB | GAGACATCCTTCCGCAAACT; TCCTTCCTGCCCATAATCA |
| | β-actin | ATGATGATATCGCCGCGCTC; CCACCATCACGCCCTGG |
| *Rattus Norvegicus* | HIF-1α | AAGTCTAGGGATGCAGCACG; AGATGGGAGCTCACGTTGTG |
| | NF-κB | AGCTCCTGTCCCAGTTCTAGC; ACTCCTGGGTCTGTGTTGTTG |
| | β-actin | TCCTTCCTGGGTATGGAATC; GCACTGTGTTGGCATAGAGG |

HIF-1α, hypoxia-inducible factor-1 α; NF-κB, nuclear factor-κB; siRNA, small interfering RNA.

as a loading control. The $2^{-\Delta\Delta Cq}$ method was used to determine fold changes (24).

*Enzyme linked immunosorbent assay (ELISA).* The human IL-1β ELISA kit (cat. no. EL10028) and the rat IL-1β ELISA kit (cat. no. A1010A0301b) were purchased from Anogen-Yes Biotech Laboratories Ltd., and BioTNT, respectively. The expression levels of IL-1β in the A549 and RLE-6TN cell culture supernatants were determined, according to the manufacturer's protocol. All experiments were performed in triplicate.

*Statistical analysis.* All data are presented as the mean ± standard error of mean of at least three experimental repeats. Mean values data showed a Gaussian distribution. Comparisons between two groups were performed using a t test. Multigroup comparisons of the means were carried out using one-way analysis of variance test. The Bonferroni correction was applied for post hoc analysis. All the statistics were analyzed GraphPad Prism 7 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*NLRP3 inflammasome is activated after BLM-treatment.* Activation of the NLRP3 inflammasome and the protein expression of HIF-1α and NF-κB in A549 and RLE-6TN cells were evaluated following treatment with BLM 24 h. The levels of proteins associated with the NLRP3 inflammasome, including NLRP3, ASC and caspase-1, were analyzed by immunofluorescence. In the BLM-treated groups, the expression levels of NLRP3, ASC and caspase-1 markedly increased in A549 and RLE-6TN cells (Fig. 1A). In addition, the results demonstrated that HIF-1α and NF-κB expression markedly increased in both cell lines (Fig. 1B and C). These data confirmed that the NLRP3 inflammasome is activated in BLM-treated alveolar epithelial cells.

*HIF-1α regulates BLM-induced activation of the NLRP3 inflammasome and the expression of NF-κB.* Next, the present study investigated the role of HIF-1α in BLM-induced activation of the NLRP3 inflammasome by transfecting A549 and RLE-6TN cells with HIF-1α siRNA. The expression of HIF-1α significantly increased when both cell lines were treated with BLM for 24 h ($P<0.05$). Furthermore, HIF-1α siRNA significantly reduced the expression of HIF-1α mRNA in the siHIF-1α group and the siHIF-1α + BLM group ($P<0.05$; Fig. 2A and B). The protein level of HIF-1α was also inhibited in both cell lines following transfection with HIF-1α siRNA. The levels of proteins associated with the NLRP3 inflammasome, including NLRP3, ASC and caspase-1, and NF-κB were notably decreased in the siHIF-1α + BLM group compared with the BLM group (Fig. 2C and D). The immunofluorescence results also demonstrated that the expression of NLRP3, ASC and caspase-1 deceased after transfection with HIF-1α siRNA (Fig. 2E). These data indicate that HIF-1α could regulate the activation of the NLRP3 inflammasome in BLM-induced ALI.

*BLM-induced activation of the NLRP3 inflammasome is modulated by NF-κB.* Subsequently, the current study aimed

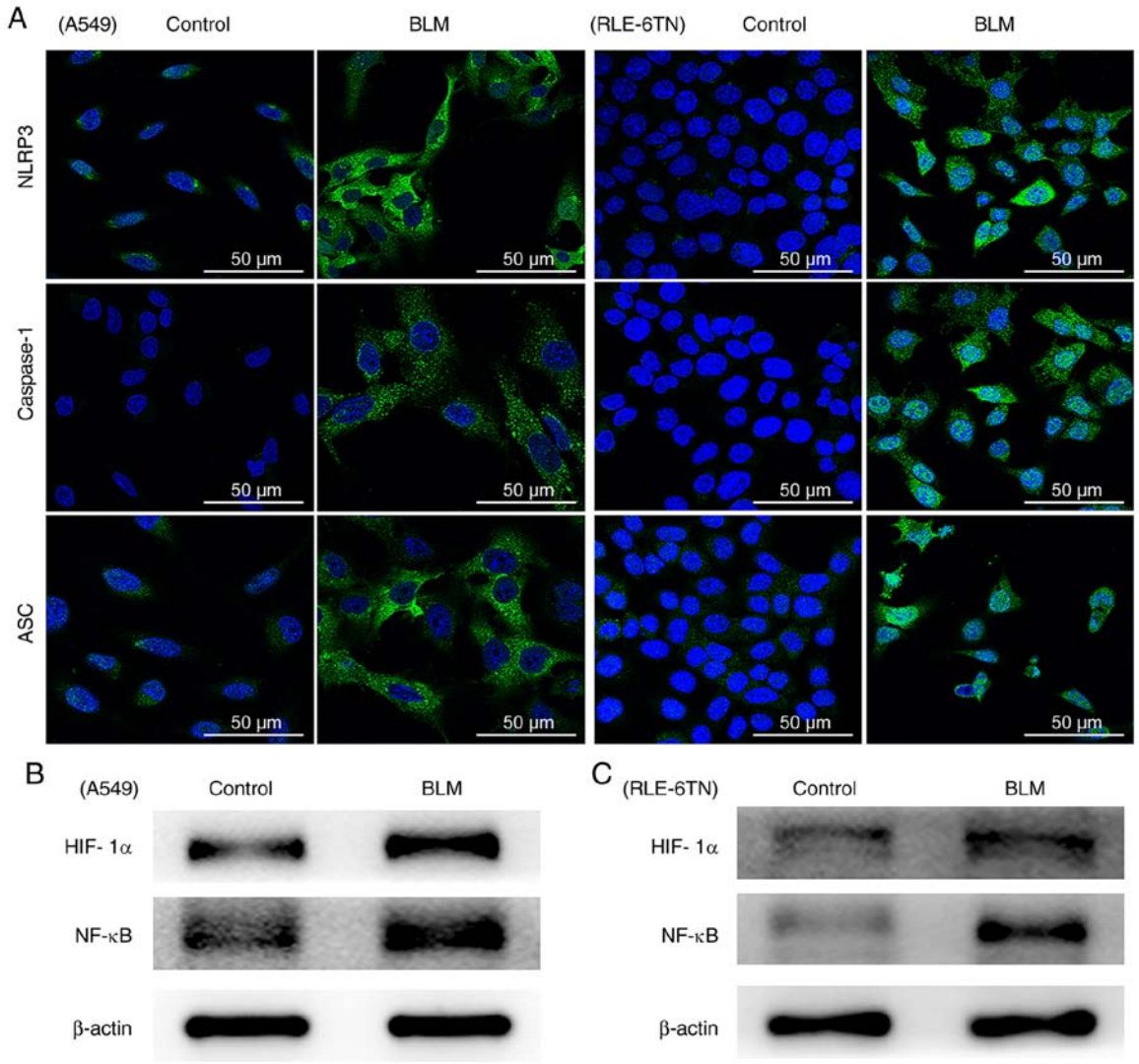


Figure 1. Changes in the NLRP3 inflammasome, HIF-1α and NF-κB after BLM-treatment. (A) Immunofluorescence for evaluating changes in the expression levels of proteins associated with the NLRP3 inflammasome, including NLRP3, ASC and caspase-1 in both cell lines. Scale bar, 50 $\mu$m. Western blotting for detecting the protein expression levels of HIF-1α and NF-κB in (B) A549 and (C) RLE-6TN cell lines after BLM-treatment. β-actin served as a loading control. NLRP3, NOD-like receptor 3; HIF-1α, hypoxia-inducible factor-1α; BLM, bleomycin; ASC, apoptosis-associated speck-like protein containing CARD; NF-κB, nuclear factor-κB.

to confirm the role of NF-κB in BLM-induced activation of the NLRP3 inflammasome by transfecting A549 and RLE-6TN cells with NF-κB siRNA. The level of NF-κB significantly increased when both cell lines were treated with BLM for 24 h ($P<0.01$). In addition, NF-κB siRNA significantly decreased the level of NF-κB mRNA in the siNF-κB group and the siNF-κB + BLM group ($P<0.05$; Fig. 3A and B). The protein level of NF-κB was also inhibited in both cell lines after transfection with NF-κB siRNA. Proteins associated with the NLRP3 inflammasome, including NLRP3, ASC and caspase-1, decreased in the siNF-κB + BLM group (Fig. 3C and D). The immunofluorescence results also demonstrated that the expression levels of NLRP3, ASC and caspase-1 deceased following transfection with NF-κB siRNA (Fig. 3E). These data indicate that NF-κB may also participate in modulating activation of the NLRP3 inflammasome in BLM-induced ALI.

*IL-1β level is regulated by HIF-1α and NF-κB*. It has been reported that IL-1β expression significantly increases after activation of the NLRP3 inflammasome (25,26). Therefore, the present study detected the levels of IL-1β in the cellular culture supernatants of A549 following inhibition of HIF-1α or NF-κB. The results demonstrated that IL-1β expression significantly increased in the BLM-treatment group ($P<0.05$) and significantly decreased in the siHIF-1α + BLM group and the siNF-κB + BLM group ($P<0.05$; Fig. 4A and B). The similar results were observed in RLE-6TN cells (Fig. 4C and D). This result indicates that BLM-induced IL-1β expression may also be regulated by HIF-1α and NF-κB.

## Discussion

ALI and ARDS, which can be caused by multiple factors, are common clinical syndromes. It has been confirmed that over-regulation of the inflammatory response is one of the most important factors leading to the occurrence and development of ALI (27,28). IL-1β is the main pro-inflammatory cytokine that may be a potential molecular biomarker for predicting morbidity

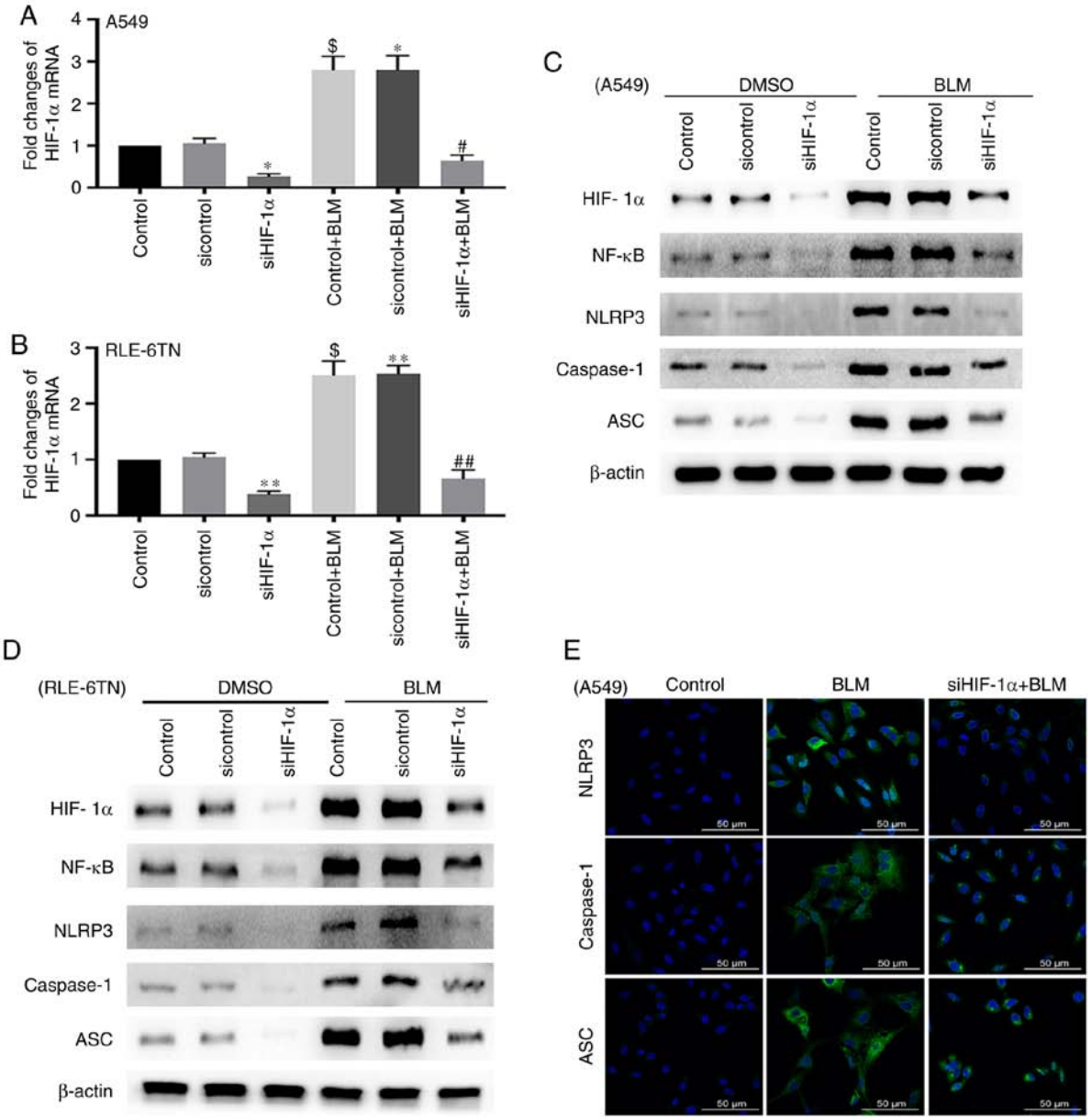


Figure 2. Expression of NF-κB and NLRP3 inflammasome-associated proteins after silencing HIF-1α. (A) RT-qPCR for detecting the mRNA expression level of HIF-1α in A549 cells after transfection with HIF-1α siRNA for 48 h and treatment with BLM for 24 h. β-actin served as a loading control. These data are presented as the mean ± standard error of the mean. $^{*}$P<0.05 [siHIF-1α vs. sicontrol; P=0.0206 (Bonferroni correction, n=2); sicontrol + BLM vs. sicontrol; P=0.0234 (Bonferroni correction, n=2), $^{\$}$P<0.05 (control + BLM vs. control; P=0.0158 (ANOVA), $^{\#}$P<0.05 (siHIF-1α + BLM vs. sicontrol + BLM; P=0.0232 (Bonferroni correction, n=2)]. (B) RT-qPCR for detecting the mRNA expression level of HIF-1α in RLE-6TN cells after transfection with HIF-1α siRNA for 48 h and treatment with BLM for 24 h. β-actin served as a loading control. These data are presented as the mean ± standard error of the mean. $^{\$}$P<0.05 [control + BLM vs. control, P=0.0141 (ANOVA)]. $^{**}$P<0.01 (siHIF-1α vs. sicontrol, P=0.0046 (Bonferroni correction, n=2); sicontrol + BLM vs. sicontrol, P=0.0038 (Bonferroni correction, n=2), $^{\#\#}$P<0.01 (siHIF-1α + BLM vs. sicontrol + BLM, P=0.0046 (Bonferroni correction, n=2). Western blotting for detecting the expression levels of HIF-1α, NF-κB and NLRP3 inflammasome-associated proteins in (C) A549 and (D) RLE-6TN cell lines after transfection with HIF-1α siRNA and treatment with BLM. β-actin served as a loading control. (E) Immunofluorescence for evaluating changes in the expression levels of proteins associated with the NLRP3 inflammasome (NLRP3, ASC and caspase-1) in A549 cells after inhibition of HIF-1α. Scale bar, 50 $\mu$m. NLRP3, NOD-like receptor 3; HIF-1α, hypoxia-inducible factor-1α; BLM, bleomycin; ASC, apoptosis-associated speck-like protein containing CARD; RT-qPCR, reverse transcription-quantitative PCR; siRNA, small interfering RNA; NF-κB, nuclear factor-κB; ANOVA, analysis of variance.

and mortality (4). The present study identified that HIF-1α may regulate activation of the NLRP3 inflammasome via NF-κB and could promote the expression of IL-1β in BLM-induced ALI.

The NLRP3 inflammasome is currently the most studied and most widely activated inflammasome. It can be activated by bacteria, fungus, virus and damage-associated molecular patterns, including uric acid crystals and silicon dioxide (29-33). It has been reported that the NLRP3 inflammasome is activated in transfusion-associated acute lung injury, ventilator-induced lung injury, asthma, chronic obstructive pulmonary disease and other pulmonary diseases. The NLRP3 inflammasome has been demonstrated to increase the release of IL-1β to participate in inflammation and the immune reaction (12). Tian *et al* (23) demonstrated that the NLRP3 inflammasome could also regulate the epithelial-mesenchymal transition in BLM-induced pulmonary fibrosis. The present study identified that the expression of

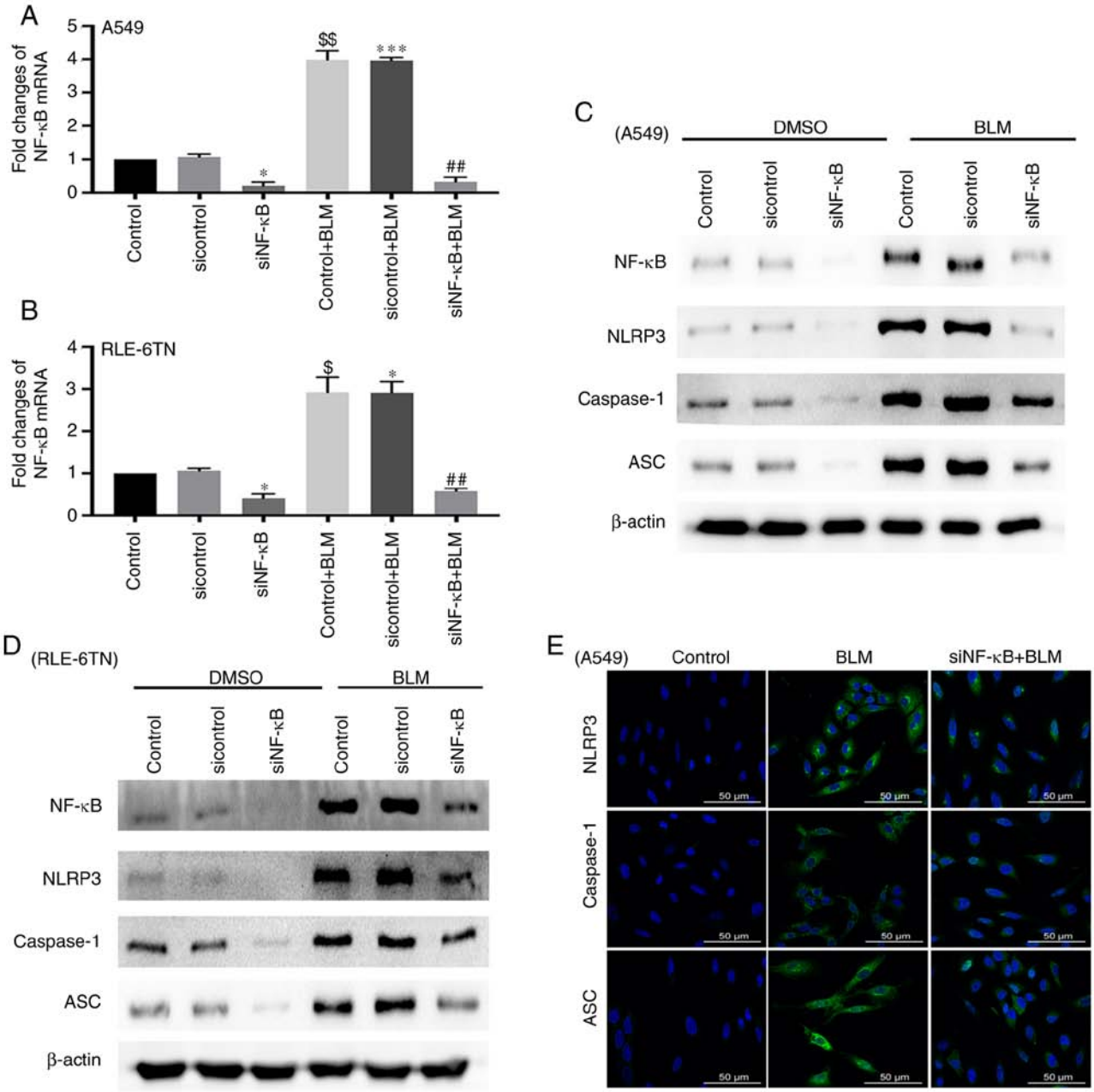


Figure 3. Expression of NLRP3 inflammasome-associated proteins after silencing NF-κB. (A) RT-qPCR for detecting the mRNA expression level of NF-κB in A549 cells after transfection with NF-κB siRNA for 48 h and treatment with BLM for 24 h. β-actin served as a loading control. These data are presented as the mean ± standard error of the mean. $^{*}$P<0.05 [siNF-κB vs. sicontrol; P=0.0246 (Bonferroni correction, n=2)], $^{***}$P<0.001 [sicontrol + BLM vs. sicontrol; P=0.0008 (Bonferroni correction, n=2)], $^{\$\$}$P<0.01 [control + BLM vs. control; P=0.0041 (ANOVA)], $^{\#\#}$P<0.01 [siNF-κB + BLM vs. sicontrol + BLM; P=0.002 (Bonferroni correction, n=2)]. (B) RT-qPCR for detecting the mRNA expression level of NF-κB in RLE-6TN cells after transfection with NF-κB siRNA for 48 h and treatment with BLM for 24 h. β-actin served as a loading control. These data are presented as the mean ± standard error of the mean. $^{*}$P<0.05 [siNF-κB vs. sicontrol; P=0.023 (Bonferroni correction, n=2); sicontrol + BLM vs. sicontrol; P=0.021 (Bonferroni correction, n=2)], $^{\$}$P<0.05 [control + BLM vs. control; P=0.0168 (ANOVA)], $^{\#\#}$P<0.01 [siNF-κB + BLM vs. sicontrol + BLM; P=0.0046 (Bonferroni correction, n=2)]. Western blotting for detecting the expression levels of NF-κB and NLRP3 inflammasome-associated proteins in (C) A549 and (D) RLE-6TN cell lines after transfection with NF-κB siRNA and treatment with BLM. β-actin served as a loading control. (E) Immunofluorescence for evaluating changes in the expression levels of proteins associated with the NLRP3 inflammasome (NLRP3, ASC and caspase-1) in A549 cells after inhibition of NF-κB. Scale bar, 50 $\mu$m. NLRP3, NOD-like receptor 3; BLM, bleomycin; ASC, apoptosis-associated speck-like protein containing CARD; RT-qPCR, reverse transcription-quantitative PCR; siRNA, small interfering RNA; siRNA, small interfering RNA; NF-κB, nuclear factor-κB.

proteins associated with the NLRP3 inflammasome, including NLRP3, ASC and caspase-1, were significantly increased in the BLM-treatment group. Therefore, it was confirmed that the NLRP3 inflammasome could be activated in BLM-induced ALI. However, the mechanism of the activation of the NLRP3 inflammasome requires further investigation.

It is understood that the body is in a state of hypoxia when ALI occurs. Numerous studies have confirmed that HIF-1α is an important regulatory factor under hypoxic conditions (34-36). In addition, a recent study demonstrated that HIF-1α could also increase under normoxic conditions (37). HIF-1α could promote the expression of inflammatory cytokines, including

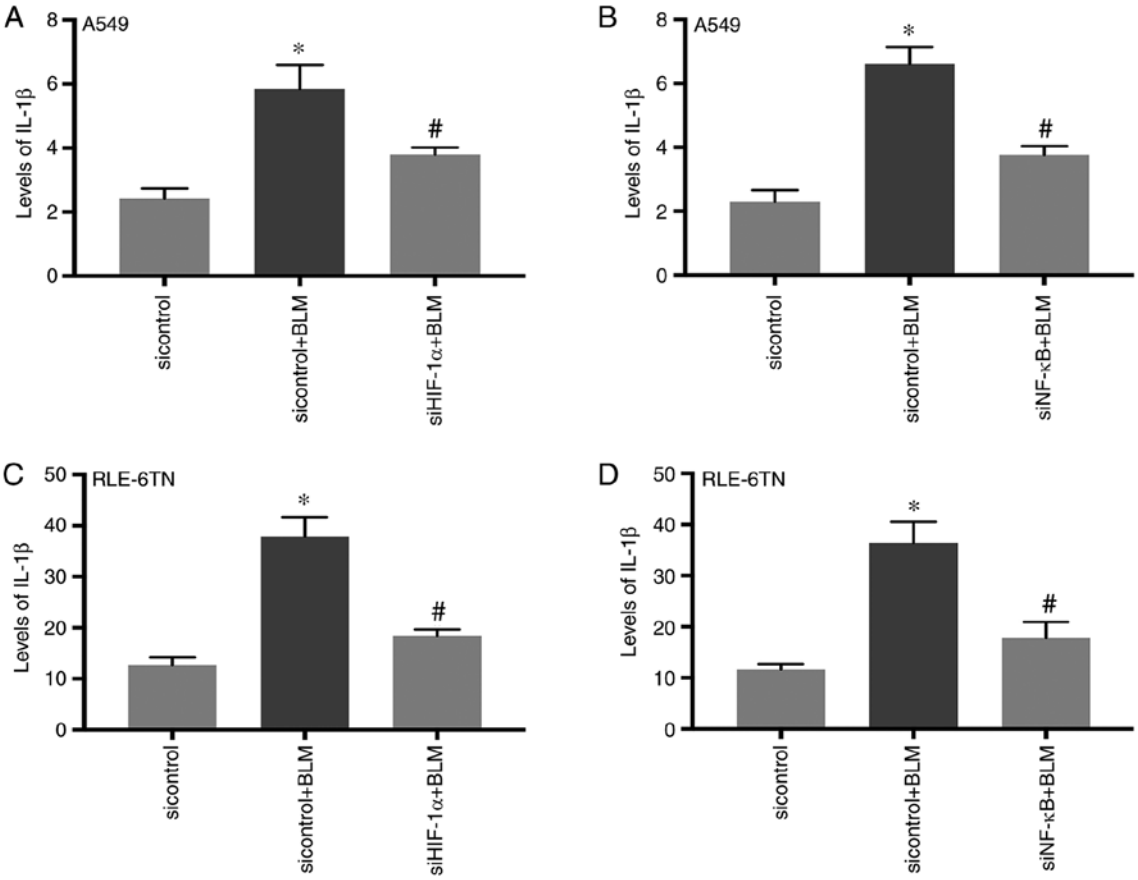


Figure 4. Levels of IL-1β in cellular culture supernatants after silencing HIF-1α or NF-κB. (A) The cellular culture supernatants of A549 cells were collected after transfection with HIF-1α siRNA. The change of IL-1β in the cellular culture supernatants was detected by ELISA. *P<0.05 [sicontrol + BLM vs. sicontrol; P=0.0354 (Bonferroni correction, n=2)], #P<0.05 [siHIF-1α + BLM vs. sicontrol + BLM; P=0.0403 (Bonferroni correction, n=2)]. (B) The cellular culture supernatants of A549 cells were collected after transfection with NF-κB siRNA. The change of IL-1β in the cellular culture supernatants was detected by ELISA. *P<0.05 [sicontrol + BLM vs. sicontrol; P=0.022 (Bonferroni correction, n=2)], #P<0.05 [siNF-κB + BLM vs. sicontrol + BLM; P=0.0432 (Bonferroni correction, n=2)]. (C) The cellular culture supernatants of RLE-6TN cells were collected after transfection with HIF-1α siRNA. The change of IL-1β in the cellular culture supernatants was detected by ELISA. *P<0.05 [siHIF-1α + BLM vs. sicontrol + BLM; P=0.0256 (Bonferroni correction, n=2)], #P<0.05 [siHIF-1α + BLM vs. sicontrol + BLM; P=0.0406 (Bonferroni correction, n=2)]. (D) The cellular culture supernatants of RLE-6TN cells were collected after transfection with NF-κB siRNA. The change of IL-1β in the cellular culture supernatants was detected by ELISA. *P<0.05 [sicontrol + BLM vs. sicontrol; P=0.029 (Bonferroni correction, n=2)], #P<0.05 [siNF-κB + BLM vs. sicontrol + BLM; P=0.0366 (Bonferroni correction, n=2)]. SiRNA, small interfering RNA; BLM, bleomycin; HIF-1α, hypoxia-inducible factor-1α; IL, interleukin.

TNF-α, IL-6 and IL-1β, in septic lymph treated A549 cells and human pulmonary microvascular endothelial cells (18). Ouyang *et al* (38) reported that HIF-1α sustains inflammasome activity via the cAMP/PKA/CREB/HIF-1α pathway in adenosine-stimulated murine peritoneal macrophages. However, whether HIF-1α can regulate the activation of the NLRP3 inflammasome in ALI has not been reported. The current study identified that HIF-1α was increased in BLM-treated A549 and RLE-6TN cells. In addition, activation of the NLRP3 inflammasome was inhibited when the expression of HIF-1α was silenced in BLM-induced ALI. These results indicate that BLM-induced activation of the NLRP3 inflammasome could be regulated by HIF-1α.

NF-κB is a transcription factor that serves an important role in regulating the transcription of multiple inflammatory factors and cytokines. A number of studies have reported that NF-κB can increase in response to multiple factors induced in ALI (39,40). In a lipopolysaccharide-treated alveolar macrophages cell line, myeloid differentiation protein 2 could regulate the activation of the NLRP3 inflammasome and IL-1β expression via the MyD88/NF-κB pathway (41). Under hypoxic conditions, NF-κB is activated by HIF-1α to participate in regulation of inflammation, cell death and angiogenesis (42). In the present study, activation of the NLRP3 inflammasome was inhibited when HIF-1α and NF-κB were silenced in BLM-treated alveolar epithelial cells. Additionally, the levels of IL-1β were markedly decreased in the cellular culture supernatants after inhibition of HIF-1α and NF-κB. Thus, these data indicate that HIF-1α may modulate the activation of the NLRP3 inflammasome and the secretion of IL-1β via NF-κB signaling.

In conclusion, it was confirmed that the NLRP3 inflammasome is activated in BLM-induced ALI. Furthermore, HIF-1α was demonstrated to modulate activation of the NLRP3 inflammasome through NF-κB and subsequently promote the expression of IL-1β. The present results promoted understanding regarding the mechanism of ALI and may provide new ideas in identifying therapeutic targets of ALI. However, the current study is limited to clinical guidance as it was primarily performed *in vitro*. In the future, further research will be performed in *in vivo* experiments and clinical research.

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**Availability of data and materials**

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

**Authors' contributions**

JH and YL conceived and designed the study, and analyzed and interpreted the results. JH performed the experiments and wrote the manuscript. JX and LH conducted the experiments. 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.

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