# N-acetyl cysteine inhibits the lipopolysaccharide-induced inflammatory response in bone marrow mesenchymal stem cells by suppressing the TXNIP/NLRP3/IL-1β signaling pathway

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**Abstract.** N-acetyl cysteine (NAC) has been used to inhibit lipopolysaccharide (LPS)-induced inflammation. However, the molecular mechanism underlying its anti-inflammatory effects remains to be elucidated. The present study aimed to determine the effect of NAC on the LPS-induced inflammatory response in bone marrow mesenchymal stem cells (BMSCs) and elucidate the underlying molecular mechanism. First, BMSCs were stimulated by LPS following pretreatment with NAC (0, 0.1, 0.5, 1 or 2 mM). A Cell Counting Kit 8 assay was used to determine the number of viable cells and 1 mM NAC was selected as the experimental concentration. Then, the secretion of inflammatory factors, including interleukin (IL)-1β, IL-6 and tumor necrosis factor-α was evaluated by enzyme-linked immunosorbent assay. Finally, the expression levels of mRNA and proteins, including apoptosis-associated speck-like protein containing a CARD (ASC), nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3), caspase-1, thioredoxin-interacting protein (TXNIP), and thioredoxin (TRX), were evaluated by reverse transcription-quantitative PCR and western blot analysis, respectively. The results demonstrated that the secretion of inflammatory factors, which was increased by the administration of LPS, was reduced by pretreatment with NAC. Furthermore, NAC reduced the expression of ASC, NLRP3, caspase-1 and TXNIP, but enhanced that of TRX. To conclude, NAC had anti-inflammatory effects on LPS-stimulated BMSCs, which was closely associated with the TXNIP/NLRP3/IL-1β signaling pathway. Thus, NAC may be a promising treatment to attenuate the inflammatory response in LPS-induced BMSCs.

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*Key words:* n-acetyl cysteine, lipopolysaccharide, bone marrow mesenchymal stem cells, nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome, interleukin-1β, inflammation

## Introduction

Oral inflammation, including periodontitis and peri-implant disease, has become a common challenge encountered in the dental clinic. It directly leads to bone resorption around the tooth or implant, subsequently causing loosening of the tooth and implant failure (1,2). There is a high prevalence of periodontitis and peri-implant disease in the United States (3,4). The National Health and Nutrition Examination Survey conducted between 2009 and 2014 reporterd that 42.2% of US dentate adults aged ≥30 years had periodontitis (5). Derks *et al* (6) found that 45% of 588 patients who had received implant-supported oral rehablitation therapy 9 years earlier presented with peri-implant disease. The principal etiological factor for these inflammatory diseases is plaque accumulation (7,8). Among the vast array of oral bacteria, *Porphyromonas gingivalis* serves a particularly important role in both periodontitis and peri-implant diseases, resulting from the expression of various virulence factors, the most recognizable of which is lipopolysaccharide (LPS) (9,10). LPS, also known as endotoxin, induces a variety of cell types, such as osteoblasts (11), human umbilical vein endothelial cells (12) and gingival fibroblasts (13), to overexpress related inflammatory factors, including interleukin (IL)-1β, IL-6 and tumor necrosis factor α (TNF-α). Recently, it was demonstrated that LPS could induce the overexpression of TNF-α, IL-1β and IL-6 in bone marrow mesenchymal stem cells (BMSCs) (14). To identify the molecular mechanism underlying LPS-mediated inflammation, the majority of studies have focused on the vital role served by nuclear factor (NF)-κB, as LPS can mediate the phosphorylation of p65, which is critical for the transactivation of NF-κB (15).

By contrast, less attention has been paid to the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome in studies on LPS-induced inflammation. LPS is widely known to stimulate the NLRP3 inflammasome in different cell types (16-18). Additionally, the NLRP3 inflammasome serves a significant role in LPS-mediated inflammation, as its upregulation is conducive

to the secretion of IL-1β and the excessive release of IL-1β contributes to the cellular inflammatory response (19-21). Thus, there is an urgent need for a novel therapeutic approach that blocks activation of the NLRP3 inflammasome and controls inflammation triggered by LPS, thereby eradicating the infection that occurs in both soft and hard tissue and eventually facilitating the development of bone around the tooth or implant.

N-acetyl cysteine (NAC), a precursor of glutathione, is a typical oxygen-free radical scavenger and anti-inflammatory agent (22). It is used as a tool for inhibiting the release of inflammatory factors and reducing the inflammation triggered by LPS (11), as well as in the treatment of different diseases (23-25), including cardiovascular diseases, respiratory diseases and cancer. In our previous studies (10,11,26), human umbilical vein endothelial cells, osteoblasts and periodontal ligament fibroblasts were pretreated with NAC prior to their stimulation with LPS. The results demonstrated that this pretreatment led to the inhibition of IL-1β, IL-6 and TNF-α, and the inflammatory response initiated by LPS.

Based on these findings, it was hypothesized that NAC may inhibit the inflammatory response in LPS-induced BMSCs. To test this hypothesis and to clarify the molecular mechanism underlying the anti-inflammatory action of NAC, the effect of NAC on inflammation stimulated by LPS was evaluated. Whether NAC could control the inflammatory response in LPS-mediated BMSCs was also assessed and the associated anti-inflammatory mechanisms were determined.

## Materials and methods

*Cell culture*. BMSCs (cat. no. BNCC340947; BeNa Culture Collection; Beijing Beina Chunglian Biotechnology Research Institute) were maintained in Dulbecco's modified Eagle's medium (DMEM; HyClone; Cytiva) supplemented with 1% (v/v) penicillin-streptomycin (Beyotime Institute of Biotechnology) and 10% (v/v) fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and then cultured at 37˚C with 5% $CO_2$. BMSCs were used from the fifth to eighth generations.

Four experimental groups were established (Table I): i) Control; ii) NAC + LPS; iii) resveratrol (Res, 50 μM; Sigma-Aldrich; Merck KGaA) + LPS; and iv) LPS. Cells in the NAC + LPS group were incubated with 1 mM NAC (Sigma-Aldrich; Merck KGaA) for 1 h, while cells in the Res + LPS group were cultured for 2 h in the dark. Next, all groups, excluding the control group, were stimulated with LPS (1 μg/ml; Sigma-Aldrich; Merck KGaA) for 24 h. All experiments were performed at 37˚C with 5% $CO_2$.

*Cell proliferation assay*. The effects of NAC on BMSC proliferation were determined by a Cell Counting Kit-8 (CCK-8) assay. First, $1x10^5/cm^2$ BMSCs were seeded into a 96-well plate and incubated for 24 h at 37˚C. Next, BMSCs were pretreated with NAC at concentrations of 0, 0.1, 0.5, 1 and 2 mM for 1 h prior to stimulation with LPS at 37˚C. The cells were then cultured for 24 h at 37˚C. Subsequently, the original medium was carefully removed prior to the addition of 10 μl CCK-8 reagent (Beyotime Institute of Biotechnology) and 100 μl DMEM to each well. Following incubation at 37˚C for 1 h, a microplate reader (Thermo Fisher Scientific, Inc.) was used to detect the absorbance of each well at 450 nm. The appropriate concentration of NAC for cell proliferation was selected with three replicates and the assay was conducted according to the manufacturer's protocols.

Table I. Experimental groups.

| Groups | Control | NAC + LPS | Res + LPS | LPS |
|---|---|---|---|---|
| NAC | - | + | - | - |
| LPS | - | + | + | + |
| Res | - | - | + | - |

NAC, N-acetyl cysteine; LPS, lipopolysaccharide; Res, resveratrol.

*Reverse transcription-quantitative (RT-q) PCR*. BMSCs ($1x10^5/cm^2$) were seeded into four plates. Subsequently, the cells were collected and total RNA of apoptosis-associated speck-like protein containing a CARD (ASC), NLRP3, caspase-1, thioredoxin-interacting protein (TXNIP) and thioredoxin (TRX) was isolated from the BMSCs using TRIzol® reagent (Thermo Fisher Scientific, Inc.). The SYBR-Green Real-Time PCR Master Mix (KAPA Biosystems; Roche Diagnostics) was used for qPCR, and cDNA synthesis was performed using the PrimeScript RT Reagent kit (Takara Bio, Inc.) in a reaction that included 1 μl cDNA, 0.5 μl forward primer, 0.5 μl reverse primer, 10 μl SYBR FAST qPCR Master Mix and 8 μl $ddH_2O$ to a total volume of 20 μl. All reactions were performed according to the manufacturer's protocols. The PCR protocol was as follows: 95˚C for 3 min, followed by 40 cycles of 95˚C for 5 sec, 56˚C for 10 sec, and 72˚C for 25 sec. Assays were conducted in triplicate and β-actin served as the reference gene. The primers are shown in Table II. Finally, the values of $2^{-\Delta\Delta Cq}$ reflected the mRNA abundance (27).

*Western blot analysis*. Western blot analysis was conducted to calculate the levels of related proteins, including ASC, NLRP3, caspase-1, TXNIP and TRX, in the BMSCs. The BMSCs were treated as aforementioned, and the total protein was collected with radioimmunoprecipitation assay buffer (Thermo Fisher Scientific, Inc.). The total protein concentration was normalized following quantification with the BCA Protein Assay (Beijing Solarbio Science & Technology Co., Ltd). The proteins (20 μg) were then separated via 12% SDS-PAGE (Sigma-Aldrich; Merck KGaA) and electrotransferred to polyvinylidene fluoride membranes. The membranes were blocked in 5% non-fat milk at room temperature for 2 h and then incubated with the following rabbit primary antibodies for ≥8 h at 4˚C: ASC (1:1,000; cat. no. PAB30696; Bioswamp), caspase-1 (1:1,000; cat. no. PAB36756; Bioswamp), TXNIP (1:1,000; cat. no. PAB43948; Bioswamp), TRX (1:1,000; cat. no. PAB32168; Bioswamp), NLRP3 (1:1,000; cat. no. PAB37930; Bioswamp) and β-actin (1:1,000; cat. no. PAB36265; Bioswamp). Subsequently, the membranes were treated at room temperature for 1 h using goat anti-rabbit IgG (1:20,000; cat. no. SAB43714; Bioswamp) labeled with horseradish peroxidase. The bands were detected by enhanced

Table II. Primers for reverse transcription-quantitative PCR.

| Gene name | Primer sequence (5'→3') | Amplicon length (bp) |
|---|---|---|
| *ASC* | F: AGCATCCAGCAAACCA<br>R: GGACCCCATAGACCTCA | 259 |
| *NLRP3* | F: CATCTTAGTCCTGCCAA<br>R: CAACAGACGCTACACCC | 94 |
| *Caspase-1* | F: TTGAAGAGCAGAAAGCA<br>R: CAGTAGGAAACTCCGAAG | 105 |
| *TXNIP* | F: CAAGGTAAGTGTGCCG<br>R: GATTCTGTGAAGGTGATGA | 105 |
| *TRX* | F: CCAACCTTTTGACCCTTT<br>R: CCCTTCTTTCATTCCCTC | 143 |
| *β-actin* | F: TAGGAGCCAGGGCAGTA<br>R: CGTTGACATCCGTAAAGAC | 110 |

F, forward; R, reverse; ASC, apoptosis-associated speck-like protein containing a CARD; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; TXNIP, thioredoxin-interacting protein; TRX, thioredoxin.

chemiluminescence (Analytik Jena AG). Tanon GIS software version 4.2 (Tanon Science & Technology Co., Ltd.) was used to determine the band intensity.

*Enzyme-linked immunoassay (ELISA).* The BMSCs were treated as aforementioned, then the supernatants were collected, which were centrifuged at 1,000 x g at room temperature for 10 min. The expression of inflammatory mediators, including IL-1β, IL-6 and TNF-α, was evaluated. All steps were in accordance with the protocols of rat IL-1β (cat. no. RLB00), IL-6 (cat. no. R6000B) and TNF-α (cat. no. RTA00) ELISA kits (R&D Systems, Inc.). The absorbance of each group was evaluated at 450 nm with a microplate reader. Finally, the concentrations of the inflammatory mediators were measured according to a standard curve.

*Statistics analysis.* All data are expressed as the mean ± standard deviation. Data analysis was performed using SPSS 17.0 (SPSS, Inc.). For multiple comparisons, the differences in variables among groups were analyzed using one-way ANOVA followed by a Tukey's post hoc test. For comparisons between two groups, one-way ANOVA was used. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Effect of NAC on the proliferation rate of LPS-mediated BMSCs.* The cytotoxicity of NAC is shown in Fig. 1A. The results demonstrated no significant changes in the viability of BMSCs following pretreatment with NAC alone at various concentrations (0, 0.1, 0.5, 1 or 2 mM) for 24 h. In order to determine the most suitable concentration for BMSC proliferation to use in subsequent experiments, BMSCs were pretreated with NAC (0, 0.1, 0.5, 1, 2 mM) and incubated for 1 h prior to stimulation with LPS for 24 h. The results are shown in Fig. 1B. An NAC concentration of 1 mM had the strongest regulatory effect on the proliferation rate of BMSCs compared with no treatment (P<0.05 vs. control group); an increase in concentration from 1 to 2 mM NAC did not lead to a significant difference [LPS + NAC (1 mM) group vs. LPS + NAC (2 mM) group, P>0.05, P=0.957]. Thus, 1 mM NAC was selected for the subsequent assays.

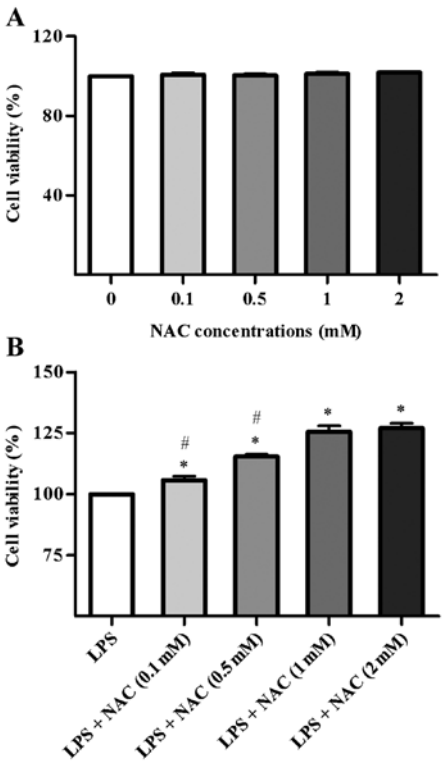


Figure 1. Effects of NAC on viability of BMSCs. (A) Effects of NAC (0, 0.1, 0.5, 1 or 2 mM) on the viability of BMSCs at 24 h. (B) BMSCs were pretreated with varying concentrations of NAC (0, 0.1, 0.5, 1 or 2 mM) for 1 h followed by treatment with LPS (1 $\mu$g/ml) for 24 h. *P<0.05 vs. LPS group; #P<0.05 vs. LPS + NAC (2 mM) group. NAC, N-acetyl cysteine; BMSCs, bone marrow mesenchymal stem cells; LPS, lipopolysaccharide.

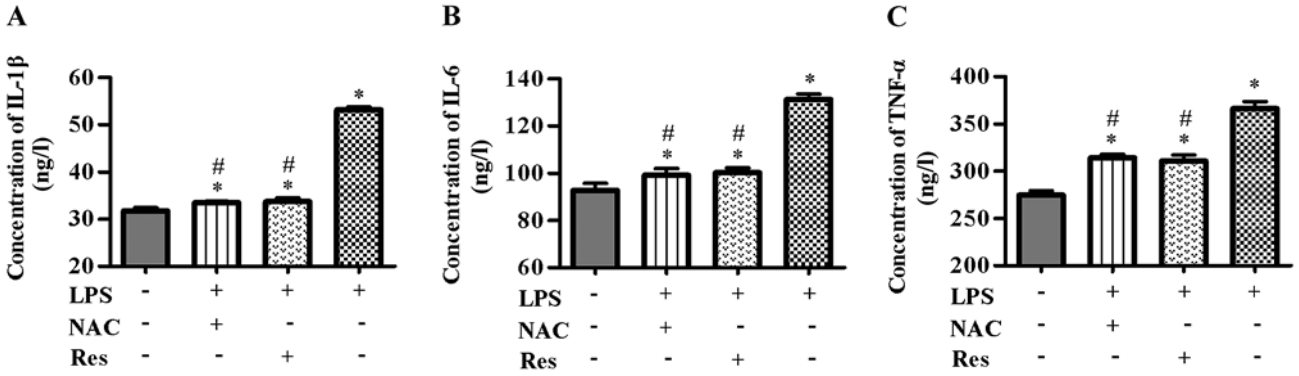


Figure 2. Effects of NAC and Res on inflammatory mediators in LPS-stimulated BMSCs. The secretion of IL-1β, IL-6 and TNF-α was evaluated by ELISA. The concentration of (A) IL-1β, (B) IL-6 and (C) TNF-α. *P<0.05 vs. control group; #P<0.05 vs. LPS group. NAC, N-acetyl cysteine; Res, resveratrol; LPS, lipopolysaccharide; BMSCs, bone marrow mesenchymal stem cells; IL, interleukin; TNF-α, tumor necrosis factor-α.

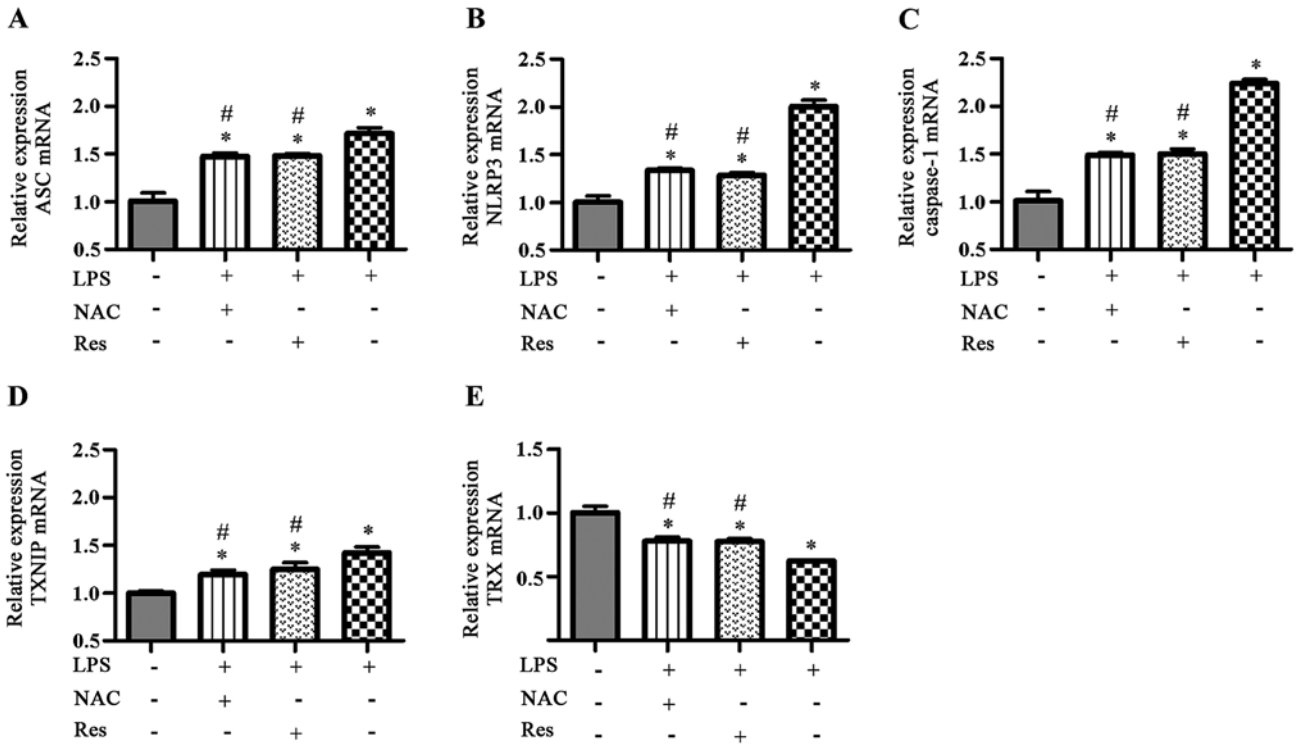


Figure 3. Effects of NAC and Res on the mRNA expression of ASC, NLRP3, caspase-1, TXNIP and TRX in LPS-stimulated BMSCs. The levels were determined by reverse transcription-quantitative PCR. The relative expression of (A) ASC, (B) NLRP3, (C) caspase-1, (D) TXNIP and (E) TRX. *P<0.05 vs. control group; #P<0.05 vs. LPS group. NAC, N-acetyl cysteine; Res, resveratrol; ASC, apoptosis-associated speck-like protein containing a CARD; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; TXNIP, thioredoxin-interacting protein; TRX, thioredoxin; LPS, lipopolysaccharide; BMSCs, bone marrow mesenchymal stem cells.

*NAC and Res decrease the levels of inflammatory mediators in LPS-stimulated BMSCs*. To investigate whether NAC could inhibit inflammation in LPS-induced BMSCs, inflammatory mediators in response to NAC or Res, which is a signaling pathway inhibitor, were detected by ELISA kits. As shown in Fig. 2, expression of IL-1β, IL-6 and TNF-α was higher in BMSCs in the LPS group compared with the control group (P<0.05). At the same time, pretreatment with NAC for 1 h led to a significant decrease in inflammatory mediators (P<0.05). Similar results were observed in the Res + LPS and NAC + LPS groups (NAC + LPS vs. Res + LPS groups, P>0.05; IL-β, P=0.980; IL-6, P=0.961; TNF-α, P=0.876). Together, the results demonstrated that LPS could induce an inflammatory response, whereas NAC or Res pretreatment clearly suppressed LPS-triggered inflammation.

*TXNIP/NLRP3 mediates the regulatory effects of NAC on inflammation in LPS-treated BMSCs*. To further identify the potential molecular mechanism underlying the regulatory effects of NAC on LPS-mediated BMSCs, RT-qPCR and western blotting were conducted to evaluate the expression of mRNA and proteins, respectively, in LPS-treated BMSCs. According to the RT-qPCR results (Fig. 3), compared with the other groups, the LPS group (P<0.05) had the highest

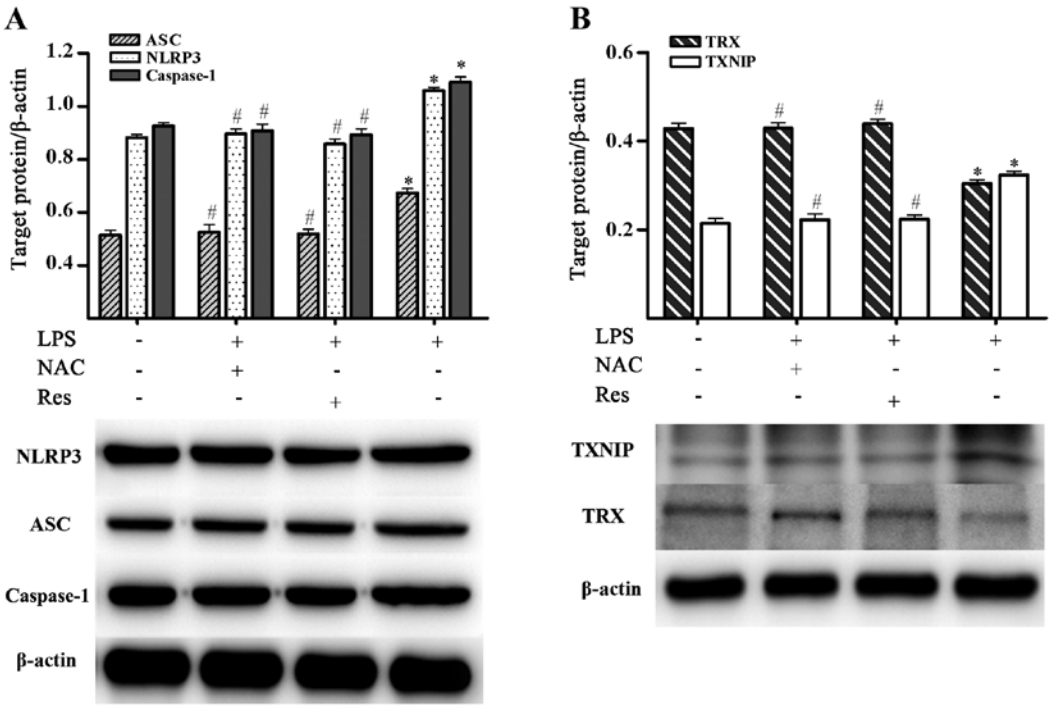


Figure 4. Effects of NAC and Res on the protein expression of ASC, NLRP3, caspase-1, TXNIP and TRX in LPS-stimulated BMSCs. Levels of proteins were measured by western blot analysis. (A) Protein expression of ASC, NLRP3 and caspase-1. (B) Protein expression of TXNIP and TRX. *P<0.05 vs. control group; #P<0.05 vs. LPS group. NAC, N-acetyl cysteine; Res, resveratrol; ASC, apoptosis-associated speck-like protein containing a CARD; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; TXNIP, thioredoxin-interacting protein; TRX, thioredoxin; LPS, lipopolysaccharide; BMSCs, bone marrow mesenchymal stem cells.

expression of ASC, NLRP3, caspase-1 and TXNIP, but the lowest expression of TRX. However, the effects of LPS on BMSCs were reversed with NAC pretreatment (P<0.05). Specifically, NAC pretreatment downregulated the expression of mRNAs increased by LPS and only upregulated the expression of TRX. In addition, when BMSCs were pretreated with Res, the same result as treatment with NAC was observed (NAC + LPS vs. Res + LPS, P>0.05; NLRP3, P=0.892; ASC, P=1.000; caspase-1, P=0.999; TXNIP, P=1.000; TRX, P=0.927). As shown in Fig. 4, the western blotting results were consistent with the mRNA data (P<0.05 vs. LPS group) (NAC + LPS vs. Res + LPS, P>0.05; NLRP3, P=0.394; ASC, P=0.998; caspase-1, P=0.954; TXNIP, P=0.912; TRX, P=1.000) (control vs. NAC + LPS, P>0.05; NLRP3, P=0.932; ASC, P=0.986; caspase-1, P=0.913; TXNIP, P=1.000; TRX, P=0.945) (control vs. Res + LPS, P>0.05; NLRP3, P=0.708; ASC, P=0.998; caspase-1, P=0.666; TXNIP, P=0.898; TRX, P=0.922). Briefly, pretreatment with NAC or Res reduced the expression of ASC, NLRP3, caspase-1 and TXNIP, but increased expression of TRX compared with cells only treated with LPS.

## Discussion

The results of the present study demonstrated that NAC inhibited the LPS-induced inflammatory response in BMSCs by significantly mediating the downregulation of IL-1β, IL-6 and TNF-α, which were upregulated in response to LPS treatment. In addition, it was identified that following treatment with LPS, NLRP3, ASC, caspase-1 and TXNIP were upregulated, but TRX was downregulated in BMSCs. It was also identified that NAC downregulated the expression of ASC, NLRP3, caspase-1 and TXNIP, but enhanced the expression of TRX in LPS-induced BMSCs, which was similar to the effect of Res. Consequently, the results suggested that LPS induced inflammatory responses in BMSCs, which could be reversed by the anti-inflammatory activity of NAC via inhibition of the TXNIP/NLRP3/IL-1β signaling pathway (Fig. 5).

Recently, BMSCs have attracted increasing attention, due to their self-renewal ability and multidirectional differentiation potential (28). BMSCs are located in alveolar bone and are strongly associated with bone formation and resorption (29). It has been confirmed that the majority of osteoblasts colonizing the implant surface are generated from BMSCs, contributing significantly to osseointegration (30,31). Meanwhile, several studies have reported that BMSCs can be effectively used for the treatment of periodontitis (14,32,33). However, the viability and osteogenic differentiation of BMSCs can be downregulated in an inflammatory environment, which directly results in treatment failure (34). Thereby, controlling inflammation is essential for diseases associated with BMSCs.

A number of studies have demonstrated that LPS-induced overproduction of IL-1β, IL-6 and TNF-α contributes to the majority of inflammatory responses that are involved in the pathogenesis of inflammatory-stimulated oral bone diseases, including periodontitis (35), peri-implantitis (36) and apical periodontitis (37). By contrast, our previous studies identified that NAC inhibited LPS-mediated synthesis of IL-1β, IL-6 and TNF-α (10,26). Thus the present study focused on the secretion of IL-1β, IL-6 and TNF-α. It was demonstrated that in BMSCs, IL-1β, IL-6 and TNF-α were overexpressed following administration of LPS, leading to generation of inflammatory

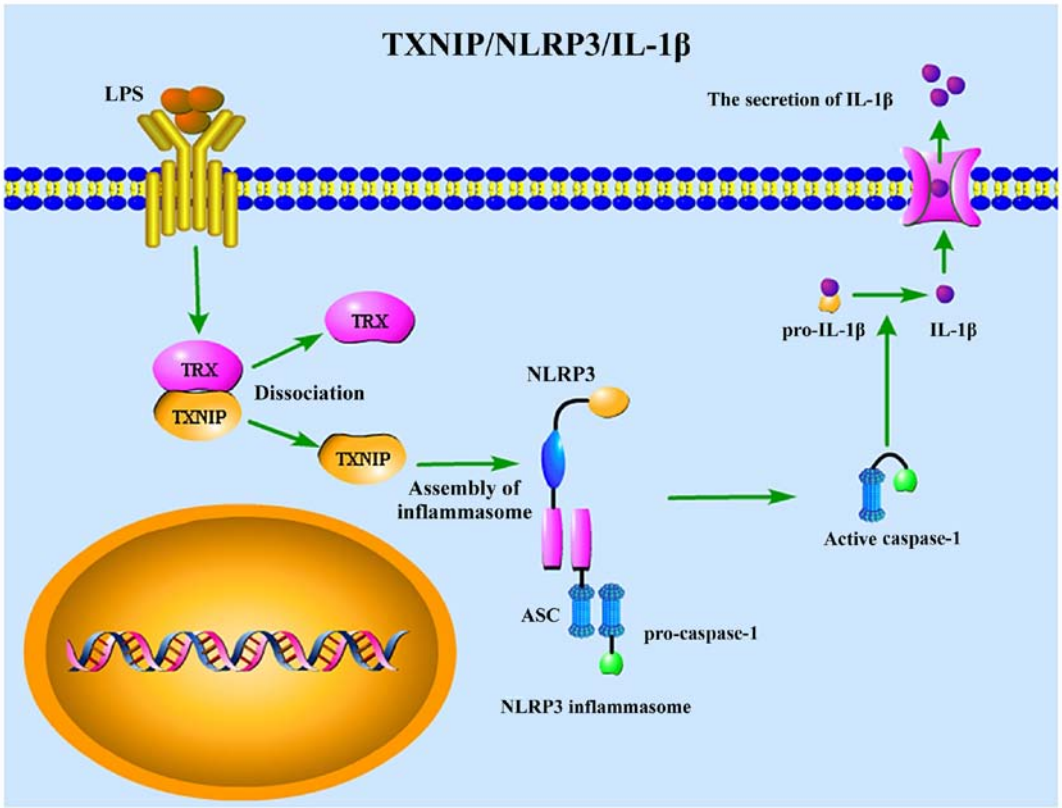


Figure 5. Mechanism of the effect of LPS on the TXNIP/NLRP3/IL-1β pathway. Following stimulation with LPS, TXNIP dissociates from TRX and then directly combines with a specific region of NLRP3. In addition, the incorporation of TXNIP and NLRP3 accelerates inflammasome aggregation and oligomerization, inducing the activation of caspase-1, which directly facilitates the maturation of IL-1β. LPS, lipopolysaccharide; TXNIP, thioredoxin-interacting protein; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; IL, interleukin; TRX, thioredoxin.

responses. Pretreatment with NAC reduced the secretion of IL-1β, IL-6 and TNF-α, and inhibited the inflammatory response. These findings revealed that NAC could be used to manage the inflammatory response.

The NLRP3 inflammasome is critical for secretion of the proinflammatory cytokine IL-1β (38). The present study demonstrated that NAC can reduce the secretion of IL-1β. Subsequent experiments determined whether NAC reduced IL-1β levels by inhibiting the NLRP3 inflammasome. The NLRP3 inflammasome, which contains NLRP3, ASC and caspase-1 (39), can initiate inflammation in response to infection of microbial products (40,41). It is widely recognized that LPS results in activation of the NLRP3 inflammasome (42-44). Once activated, NLRP3 interacts with ASC, inducing the reciprocal identification of NLRP3 and pro-caspase-1, thereby leading to activation of caspase-1 (45,46). Immediately, caspase-1, also termed inflammatory caspase, mediates the maturation and secretion of IL-1β (47,48), which serves a significant role in inflammation (49,50). In the present study, protein and mRNA expression levels indicated that LPS mediated overexpression of the NLRP3 inflammasome in BMSCs. However, pretreatment with NAC attenuated the expression of mRNA and target proteins. These results clearly demonstrated that NAC downregulated the NLRP3 inflammasome and inhibited inflammatory responses caused by LPS.

The specific molecular mechanism by which NAC acts on the NLRP3 inflammasome and exhibits an anti-inflammatory effect remains to be elucidated. TXNIP is an essential multi-functional protein, which interferes with the function of TRX (51). The overexpression of TXNIP activates inflammatory pathways, in part since TRX-mediated inhibition of inflammation is reversed by TXNIP (51). Additionally, TXNIP directly activates the NLRP3 inflammasome, further stimulating inflammation (52-54). Previous studies have suggested that, in response to stimulation, TXNIP dissociates from TRX and then directly combines with a specific region of NLRP3 (55,56). In addition, the incorporation of TXNIP and NLRP3 accelerates inflammasome aggregation and oligomerization, inducing transformation of caspase-1 and maturation of IL-1β (57). In the present study, LPS treatment induced the overproduction of TXNIP and decreased expression of TRX at the mRNA and protein levels. However, the expression of TXNIP was downregulated and that of TRX was upregulated by pretreatment with NAC. These observations further indicated that TXNIP and TRX are involved in the anti-inflammatory effect of NAC on LPS-induced BMSCs.

To clarify the underlying signaling pathway, Res was used as a pathway blocker. Previous studies have indicated that Res, a natural plant polyphenolic compound, can restrain NLRP3 inflammasome activation, which prevents the over-release of inflammatory cytokines (58,59). It was reported that Res can significantly inhibit the TXNIP/TRX cascade, restrain the expression of TXNIP, inhibit the incorporation of TXNIP and NLRP3 and finally block the inflammasome aggregation and oligomerization (60). As expected, in the present study, the effect of NAC was identified to be consistent with that of

Res. Therefore, it was concluded that the inflammatory regulatory effects of NAC on LPS-induced BMSCs were closely associated with the TXNIP/NLRP3/IL-1β pathway.

On one hand, the results of the present study provided novel evidence that NAC can inhibit the inflammatory response in BMSCs stimulated by LPS; on the other hand, it demonstrated that one of the molecular mechanisms underlying the inflammatory effects of NAC on LPS-induced BMSCs was closely associated with the TXNIP/NLRP3/IL-1β pathway. These findings not only broadened our view of how NAC affects LPS-induced BMSCs but also provided an improved understanding of the underlying molecular mechanism that may promote the development of anti-inflammation strategies. However, *in vivo* experiments are required to validate these findings so we plan to establish animal models to further explore the anti-inflammatory effects of NAC in future research.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

XW and LG were primarily responsible for the research protocol design. XW and MJ performed the experiments. XW, XH, BZ and WP analyzed and interpreted the data. XW drafted the manuscript. XW, LG and XH revised 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.

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