Am J Transl Res 2019;11(9):5944-5955
www.ajtr.org /ISSN:1943-8141/AJTR0099929

Original Article

Long non-coding RNA NEAT1/miR-193a-3p regulates LPS-induced apoptosis and inflammatory injury in WI-38 cells through TLR4/NF-κB signaling

Weixin Nong

Department of Emergency Intensive Care Unit, Guigang City People's Hospital, Guigang 537100, Guangxi, China

Received July 21, 2019; Accepted July 26, 2019; Epub September 15, 2019; Published September 30, 2019

Abstract: Pneumonia is a primary pulmonary infection disease with a high morbidity and mortality worldwide. Identification of key long non-coding RNAs (lncRNAs) facilitates to the development of effective therapeutic targets for pneumonia. LncRNA NEAT1 was vital and functional in inflammatory diseases but has not been studied in pneumonia. The aim of this study was to investigate the role of NEAT1 in pneumonia and explore its potential mechanism. Lipopolysaccharide (LPS) was applied into WI-38 cells to establish cell model of pneumonia. Cells were transfected with shRNA-NEAT1, miR-193a-3p or negative control. Real time quantitative PCR and western blot were performed to detect mRNA level and protein expression, respectively. Cell counting kit-8 (CCK-8) assay was performed to detect cell viability. Flow cytometry analysis was performed to determine cell apoptosis. Cell viability was significantly declined and cell apoptosis was increased in LPS-treated WI-38 cells. NEAT1 was upregulated under LPS treatment and NEAT1 inhibition significantly improved cell viability, decreased cell apoptosis and the production of inflammatory cytokines. The expression level of miR-193a-3p was regulated by NEAT1, and NEAT1 reversed miR-193a-3p overexpression-alleviated inflammatory injury that include inflammation and apoptosis induced by LPS. Further, NEAT1 and miR-193a-3p regulated the activity of Toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-κB) signaling. Therefore, NEAT1 may function as a ceRNA by sponging miR-193a-3p to regulate the activation of TLR4/NF-κB signaling to alleviate inflammation and apoptosis of WI-38 cells induced by LPS, thus influencing the development of pneumonia. Our findings implied that NEAT1 might serve as a neoteric therapy target for pneumonia.

Keywords: NEAT1, miR-193a-3p, inflammation, apoptosis, Toll-like receptor 4, nuclear factor kappa B

Introduction

Pneumonia is the acute inflammation of the lower respiratory tract and lung parenchyma due to infectious agent and clinically characteraized as chills, fever, pleuritic chest pain, and cough productive of purulent sputum [1]. Currently, pneumonia is a major global reason for mortality and morbidity all over the world, in paticular in children and elderly people [2]. Lipopolysaccharide (LPS) is a key component of the cell wall of gram-negative bacterial that is critial for the inflammatory response and induces pulmonary infection [3, 4]. Thus, LPS has been widely used to establish pulmonary inflammation related diseases. Therefore, it is urgent to explore the underlying pathogenesis and discover effective therapetic target for the treatment of pneumonia.

There are some important non-coding RNAs, including long non-coding RNA (lncRNAs) and short non-coding RNAs (microRNAs, miRNAs). lncRNAs are transcriptional RNA molecules with more than 200 nucleotides, and play an important role in biological process such as transcription, translation and cellular differentiation [5]. Previous studies showed that dysregulation of lncRNAs was closely connected with inflammation-related diseases, such as pneumonia [6]. Feng C et al analyzed the expression profile of lncRNAs from samples of pneumonia patients using mRNA-sequence and bioinformatics analysis, and identified some potential key genes associated with severe pneumonia, indicating that lncRNAs were actually involved in the progress of pneumonia and are expected to diagnosed markers for pneumonia [7]. Nuclear enriched

abundant transcript 1 (NEAT1), a nuclear-restricted lncRNA, has been demonstrated to be a key transcriptional regulator in cancer cell growth [8]. Besides, NEAT1 was also reported in many inflammatory diseases. NEAT1 could promote inflammatory response with the production of TNF-α and IL-6 and induce corneal neovascularization [9]. It was also reported that NEAT1 was dysregulated in sepsis patients. Inhibition of NEAT1 suppressed the production of cytokines and oxidative stress in LPS-induced kidney injury mice [10]. However, it has been never reported in pneumonia concerning on NEAT1.

miRNAs, about 22 nucleotides, act as an important regulator in gene expression. miRNAs are also involved in pathological process of various diseases, including pneumonia [11, 12]. MiR-193a-3p, a member of miR-193 family, has been widely reported in various cancers such as lung cancer and renal cell cancer due to its anti-tumor activity [13, 14]. Current study indicated that lncRNAs could act as competing endogenous RNA (ceRNA) and competitively bind to miRNAs, thus regulating the transcription level of mRNA and modulating gene expression [15]. It is reported that NEAT1 act as ceRNA and bind to miR-193a-3p to accelerate deterioration of lung adenocarcinoma [16]. Howerver, whether NEAT1 worked with miR-193a-3p in pneumonia is unclear.

In this study, WI-38 cell were dealt with LPS to construct pulmonary injury model. We investigated the role of NEAT1/miR-193a-3p in this model and appraised the potential molecular mechanism of them on affecting inflammarory injury. This study might provide a neoteric insight for pneumonia therapy strategy.

Materials and methods

Cell culture and treatment

Normal human fibroblast cell line WI-38 cell was obtained from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St Louis, USA) containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1% penicillin (100 U/mL) and streptomycin (100 mg/mL) (Thermo Scientific, Waltham, MA, USA). Cells were stimulated with LPS of different concentrations (0, 2.5, 5 and 10 μg/mL) for 12, 24 and 48 h, respectively.

Cell transfection

For knockdown and overexpression of NEAT1, pcDNA-NEAT1 and short hairpin RNA for targeting NEAT1 (sh-NEAT1; GenePharma, Roche, China) were transfected into WI-38 cells using Lipofectamine 2000 (Invitrogen). Besides, miR-193a-3p mimics and mimic control (GenePharma, Roche, China) were transfected into WI-38 cells to regulate expression of miR-193a-3p. After transfection for 48 h, the efficacy of transfection was detected by RT-qPCR.

Cell counting kit-8 (CCK-8) assay

After transfection, cells were seeded in 96-well plates. After 12 h, 24 h and 48 h of LPS treatment, cells were incubated with CCK-8 reagent (Beyotime, Shanghai, China), respectively. The absorbance (OD) of 450 nm was detected with a microplate reader.

Flow cytometry

After transfection, cells were treated with LPS for 24 h. Subsequently, cells were harvested, washed twice with PBS and then resuspended in 100 μL binding buffer, followed by the addition of 5 μL Annexin V-FITC and PI. After incubation in the dark room for 15 min, an additional binding buffer was added prior to analysis by flow cytometry using FACScan flow cytometer (BD Bioscience, Frankin Lakes, NJ, USA).

Western blot

Cells were lysed in lysis buffer, and the extracted protein was quantified with the BCA protein assay kit (Beyotime Nanjing, China). The same amount of protein was separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% non-fat milk, the membranes were incubated with primary antibodies at 4°C overnight. After incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies, the protein bands were visualized by ECL assay (Millipore, Billerica, MA, USA).

Real time quantitative PCR (RT-qPCR)

Total RNA was extracted from cells using Trizol Reagent (Invitrogen, CA, USA). The first cDNA of total RNA was synthesized according to the

instructions of PrimeScript™ RT reagent Kit (Takara, Dalian, China). Real time quantitative PCR was performed according to the instructions of SYBR Green qPCR kit (Thermo Scientific, Waltham, MA, USA). GAPDH and U6 was used as the endogenous control. The relative fold expression of the target was calculated by the comparative Ct method and normalized to the control.

Enzyme-linked immune sorbent assay (ELISA)

The levels of inflammatory cytokines: IL-6, IL-8, IL-1β and TNF-α and oxidative stress factor: NO and iNOS were determined by mouse ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

All data was expressed as mean ± SD and analyzed using Graphpad Prism version 6.0 and SPSS version 18.0. One-way analysis of variance (ANOVA) was used to determine the difference among groups. A p value <0.05 was considered as statistically significant.

Results

LPS decreased cell viability and increased NEAT1 expression

First, LPS was applied for WI-38 cells to cause pulmonary inflammation that has similarities with pneumonia. 0 μg/ml, 2.5 μg/ml, 5 μg/ml and 10 μg/ml of LPS was used to treat cells, respectively. CCK-8 assay showed that cell viability was dependent on the concentration and treatment time of LPS (**Figure 1A**). 10 μg/ml LPS decreased cell viability for nearly 50%. The mRNA level of NEAT1 exhibited a significant increase following the increase of LPS concentration (**Figure 1B**).

Downregulation of NEAT1 alleviated LPS-induced cell viability and apoptosis

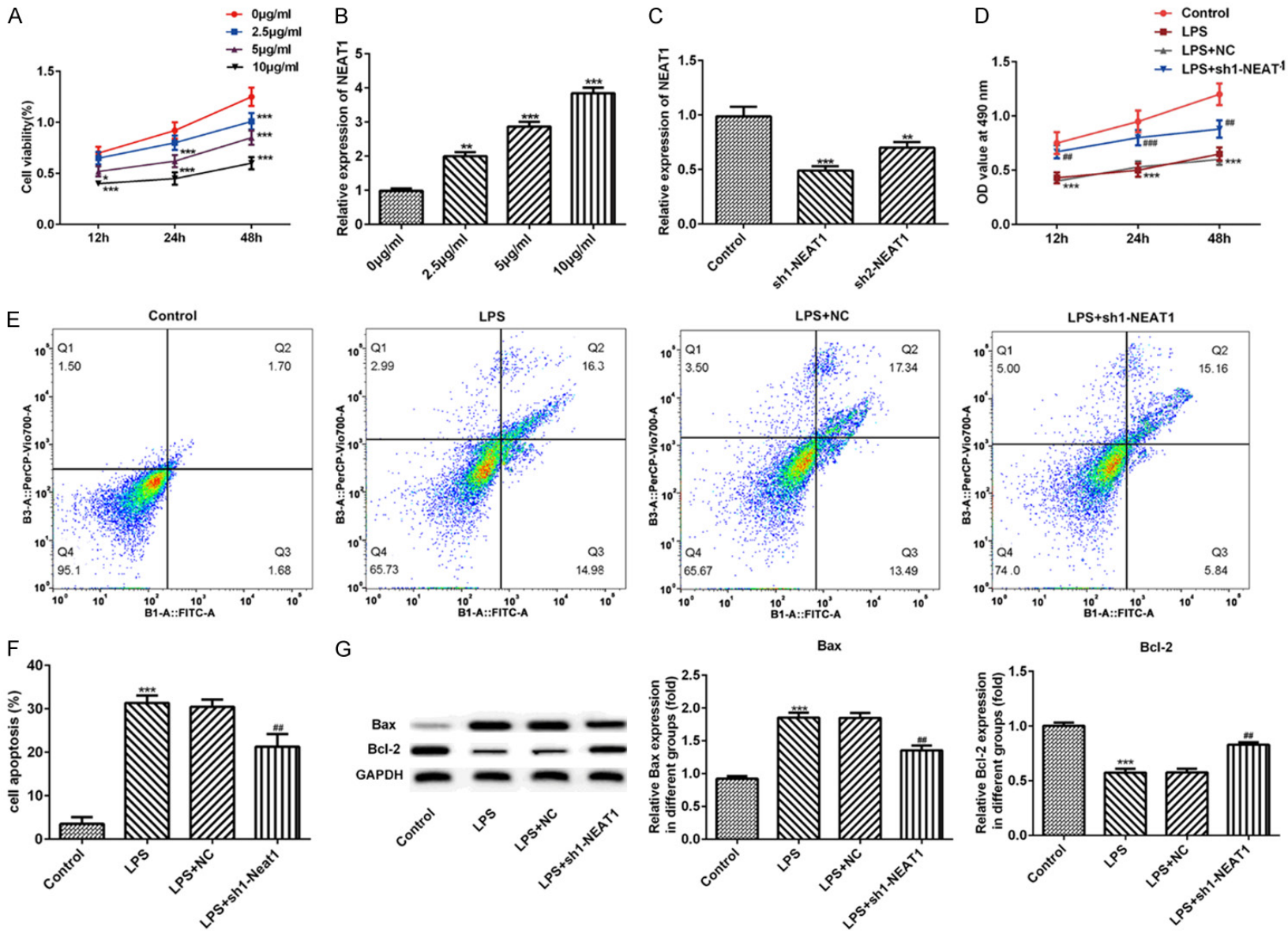
Then, sh-NEAT1 was transfected into WI-38 cells to knockdown the expression of NEAT1. The transfection efficacy was detected by RT-qPCR, and the results showed a significantly decreased expression of NEAT1, especially with sh1-NEAT1 (**Figure 1C**). Thus sh1-NEAT1 was applied for the next experiments. After transfection, cell viability was improved significantly which was decreased under LPS treatment (**Figure 1D**). Downregulation of NEAT1 also decreased cell apoptosis which was induced by LPS (**Figure 1E**, **1F**). Besides, apoptosis-related proteins that include Bax, Bcl-2, cleaved caspase-3, caspase-3, cleaved caspase-9, caspase-9, were detected by western blot (**Figure 1G-I**), and the results showed that LPS significantly decreased the expression of Bcl-2, and increased the expression of Bax, cleaved caspase-3 and cleaved caspase-9, which were then reversed when NEAT1 was downregulated. Taken together, these results suggested that NEAT1 knockdown play a protective role on LPS-induced cell apoptosis.

Downregulation of NEAT1 alleviated LPS-induced inflammatory injury

The effect of NEAT1 on LPS-induced inflammatory injury was investigated. Results in **Figure 2A-D** showed that LPS increased the expression of IL-6, IL-8, TNF-α and IL-1β, meaning a severe inflammatory response induced by LPS, while downregulation of NEAT1 effectively decreased these inflammatory cytokines. During inflammation, NO was generated mainly due to inducible NO synthase (iNOS). Results in **Figure 2E**, **2F** showed that NO production and iNOS expression was significantly increased in LPS-induced WI-38 cells, and downregulation of NEAT1 also effectively decreased the increased NO and iNOS induced by LPS. Taken together, these results suggested that NEAT1 knockdown play a protective role on LPS-induced inflammatory injury.

NEAT1 regulated LPS-induced cell apoptosis through miR-193a-3p

As mentioned above that NEAT1 acts as ceRNA and bind to miR-193a-3p to accelerate deterioration of lung adenocarcinoma, whether NEAT1 worked with miR-193a-3p in pneumonia was explored. As shown in **Figure 3A**, miR-193a-3p was upregulated when NEAT1 was inhibited, and miR-193a-3p was downregulated when NEAT1 was overexpressed, thus NEAT1 was likely to regulate the expression of miR-193a-3p. LPS treatment significantly decreased the expression of miR-193a-3p (**Figure 3B**), indicating an involvement of miR-193a-3p in LPS-induced cell injury. Thus mimics targeting miR-193a-3p was transfected into WI-38 cells to make miR-193a-3p overexpressed (**Figure**


A
0μg/ml
2.5μg/ml
5μg/ml
10μg/ml
Cell viability(%)
12h
24h
48h
B
Relative expression of NEAT1
0μg/ml
2.5μg/ml
5μg/ml
10μg/ml
C
Relative expression of NEAT1
Control
sh1-NEAT1
sh2-NEAT1
D
Control
LPS
LPS+NC
LPS+sh1-NEAT1
OD value at 490 nm
12h
24h
48h
E
Control
LPS
LPS+NC
LPS+sh1-NEAT1
Q1
Q2
Q3
Q4
1.50
1.70
95.1
1.68
2.99
16.3
65.73
14.98
3.50
17.34
65.67
13.49
5.00
15.16
74 .0
5.84
B3-A::PerCP-Vio700-A
B1-A::FITC-A
F
cell apoptosis (%)
Control
LPS
LPS+NC
LPS+sh1-Neat1
G
Bax
Bcl-2
GAPDH
Control
LPS
LPS+NC
LPS+sh1-NEAT1
Bax
Relative Bax expression in different groups (fold)
Control
LPS
LPS+NC
LPS+sh1-NEAT1
Bcl-2
Relative Bcl-2 expression in different groups (fold)
Control
LPS
LPS+NC
LPS+sh1-NEAT1

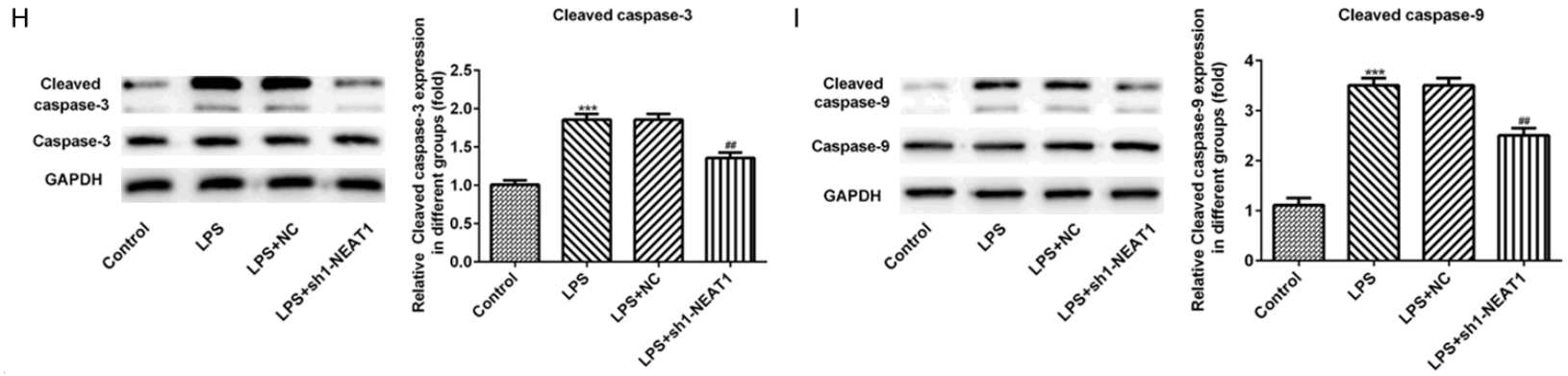


Figure 1. Downregulated NEAT1 alleviated cell viability and apoptosis induced by LPS. WI-38 cells were treated with LPS (0 μg/ml, 2.5 μg/ml, 5 μg/ml and 10 μg/ml) for 12 h, 24 h and 48 h, respectively. Cell viability was determined by CCK-8 assay (A). mRNA level of NEAT1 was determined by RT-qPCR (B). After transfection, the efficacy was detected by RT-qPCR (C), and cell viability was determined (D). Cell apoptosis was analyzed by flow cytometry and quantified (E, F). Besides, the apoptosis-related protein expressions were determined by western blot, such as Bax, Bcl-2, Cleaved caspase-3, caspase-3, Cleaved caspase-9 and caspase-9 (G-I). Data were expressed as mean ± SD. *, ***P<0.05, 0.001 compared with control. ##, ###P<0.01, 0.001 compared with LPS.

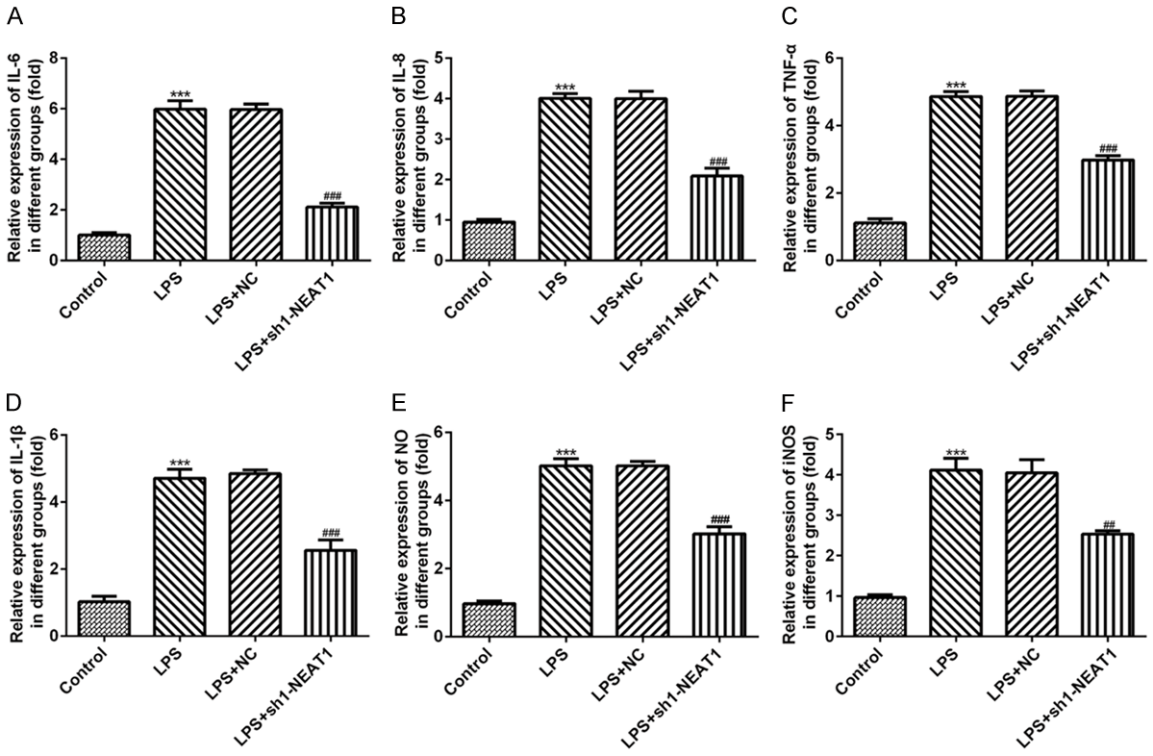


Figure 2. Downregulated NEAT1 alleviated cell inflammatory injury induced by LPS. The expressions of pro-inflammatory cytokines (IL-6, IL-8, TNF-α, and IL-1β) were measured by ELISA (A-D). The expressions of NO and inducible NO synthase (iNOS) were measured by ELISA (E, F). Data were expressed as mean ± SD. ***P<0.001 compared with control. ##, ###P<0.01, 0.001 compared with LPS.

3C). Overexpressed miR-193a-3p improved cell viability which was decreased by LPS, while upregulated NEAT1 could reverse the effect that miR-193a-3p made significantly (**Figure 3D**). Flow cytometry analysis showed that overexpressed miR-193a-3p decreased cell apoptosis, which could be reversed by upregulated NEAT1 (**Figure 3E**, **3F**). Meanwhile, apoptosis-related proteins were also detected. Overexpressed miR-193a-3p increased the expression of Bcl-2, and decreased the expression of Bax, cleaved caspase-3 and cleaved caspase-9 in LPS-induced WI-38 cells, while the changes were also reversed by upregulated NEAT1 (**Figure 3G-I**). Taken together, these results suggested that NEAT1 could regulate miR-193a-3p, and NEAT1 regulated LPS-induced cell viability and apoptosis partly through miR-193a-3p.

NEAT1 regulated LPS-induced inflammatory injury through miR-193a-3p

Next, we explored whether NEAT1 worked with miR-193a-3p in LPS-induced inflammatory injury. The detection of relative inflammatory cytokines showed that overexpressed miR-193a-3p decreased the elevated production of IL-6, IL-8, TNF-α and IL-1β induced by LPS, and upregulated NEAT1 reversed the effect that miR-193a-3p caused (**Figure 4A-D**). Meanwhile, overexpressed miR-193a-3p decreased the elevated expression of NO and iNOS that induced by LPS, which was also reversed by upregulated NEAT1 (**Figure 4E**, **4F**). Taken together, these results suggested that NEAT1 regulated LPS-induced inflammatory injury partly through miR-193a-3p.

NEAT1/miR-193a-3p regulated TLR4/NF-κB signaling pathway

The activation of Toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-κB) signaling pathway has been widely studied on its involvement in inflammatory response and cell apoptosis, we then detected the effect of NEAT1 and miR-193a-3p on TLR4/NF-κB signaling pathway proteins in LPS-induced WI-38 cells. Both RT-qPCR and western blot analysis showed that down-

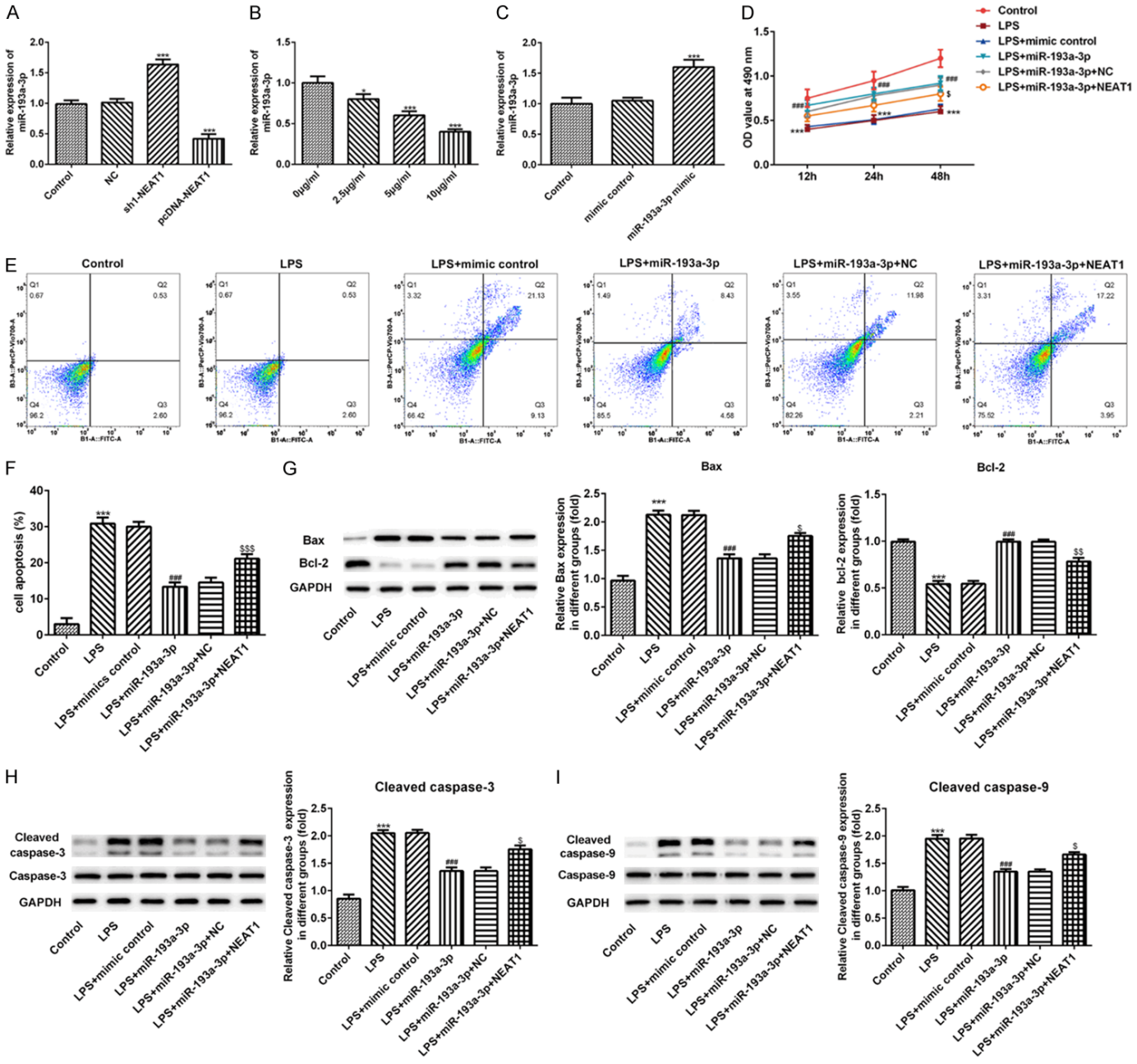

A
Relative expression of miR-193a-3p
Control
NC
sh1-NEAT1
pcDNA-NEAT1
B
Relative expression of miR-193a-3p
0μg/ml
2.5μg/ml
5μg/ml
10μg/ml
C
Relative expression of miR-193a-3p
Control
mimic control
miR-193a-3p mimic
D
OD value at 490 nm
12h
24h
48h
Control
LPS
LPS+mimic control
LPS+miR-193a-3p
LPS+miR-193a-3p+NC
LPS+miR-193a-3p+NEAT1
E
Control
LPS
LPS+mimic control
LPS+miR-193a-3p
LPS+miR-193a-3p+NC
LPS+miR-193a-3p+NEAT1
F
cell apoptosis (%)
Control
LPS
LPS+mimics control
LPS+miR-193a-3p
LPS+miR-193a-3p+NC
LPS+miR-193a-3p+NEAT1
G
Bax
Bcl-2
GAPDH
Relative Bax expression in different groups (fold)
Relative bcl-2 expression in different groups (fold)
H
Cleaved caspase-3
Caspase-3
GAPDH
Relative Cleaved caspase-3 expression in different groups (fold)
I
Cleaved caspase-9
Caspase-9
GAPDH
Relative Cleaved caspase-9 expression in different groups (fold)

Figure 3. NEAT1 regulated cell viability and apoptosis through miR-193a-3p. The expression change of miR-193a-3p under different NEAT1 expression (A) and different LPS treatment (0 μg/ml, 2.5 μg/ml, 5 μg/ml and 10 μg/ml) (B) was detected by RT-qPCR, respectively. miR-193a-3p expression after mimic transfection was detected by RT-qPCR (C). After transfection of miR-193a-3p and NEAT1 into WI-38 cells, cell viability was detected by CCK-8 assay (D), and cell apoptosis was detected by flow cytometry (E, F). Besides, the apoptosis-related protein expressions were determined by western blot, such as Bax, Bcl-2, Cleaved caspase-3, caspase-3, Cleaved caspase-9 and caspase-9 (G-I). Data were expressed as mean ± SD. *, ***P<0.05, 0.001 compared with control. ###P<0.001 compared with LPS. $, $$, $$$P<0.05, 0.01, 0.001 compared with LPS + miR-193a-3p.

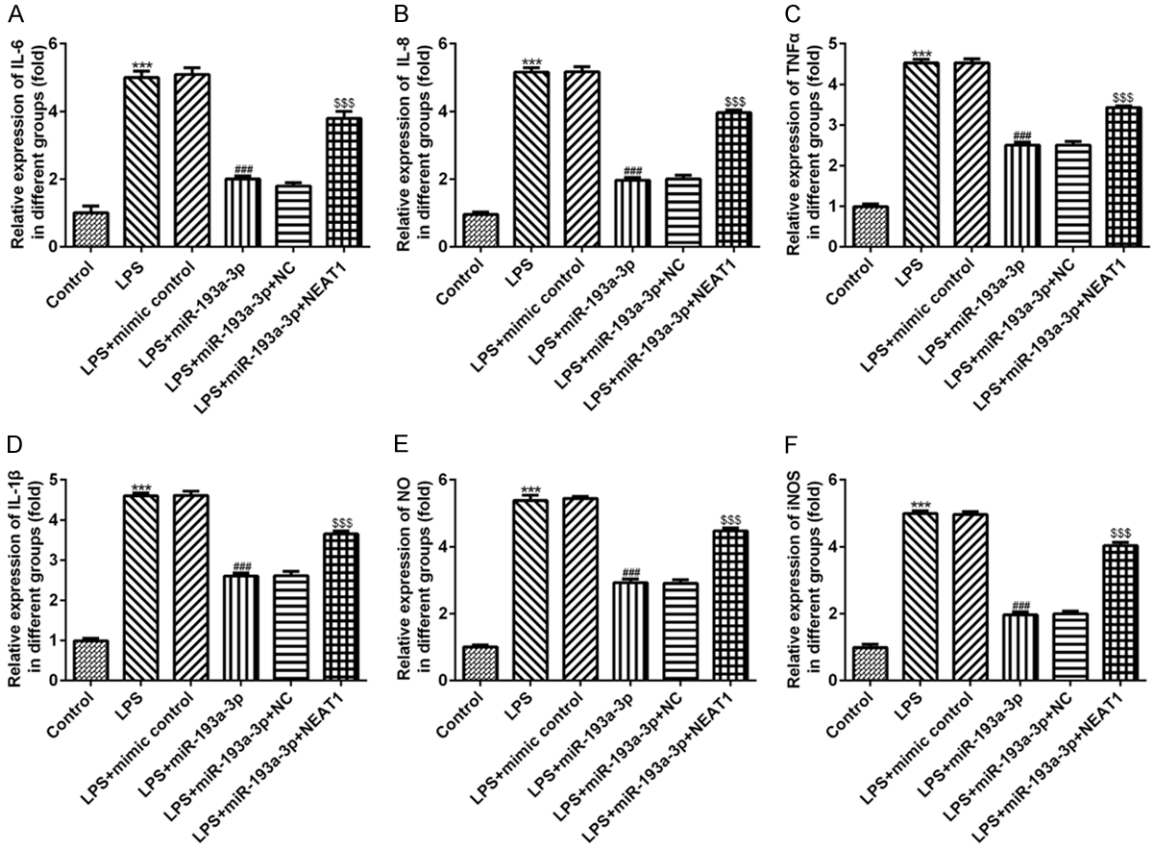


Figure 4. NEAT1 regulated LPS-induced inflammatory injury through miR-193a-3p. The expressions of pro-inflammatory cytokines (IL-6, IL-8, TNF-α, and IL-1β) were measured by ELISA (A-D). The expressions of NO and inducible NO synthase (iNOS) were measured by ELISA (E, F). Data were expressed as mean ± SD. ***P<0.001 compared with control. ###P<0.001 compared with LPS. $, $$, $$$P<0.05, 0.01, 0.001 compared with LPS + miR-193a-3p.

regulated NEAT1 and overexpressed miR-193a-3p decreased the mRNA level and protein expression of TLR4, myeloid differential protein-88 (MyD88) and p-p65. Besides, upregulated NEAT1 could also reversed the genes expression that caused by overexpressed miR-193a-3p (**Figure 5A-D**). Taken together, these results suggested that NEAT1 could regulate TLR4/NF-κB signaling pathway partly through miR-193a-3p.

Discussion

Pneumonia is a primary pulmonary infection disease with a high morbidity and mortality worldwide, especially in children and elder. Identification of key lncRNAs facilitates to the development of effective therapeutic targets for pneumonia. In the present study, LPS was applied for establishment of cell model to explore the underlying mechanism involved in pneumonia. NEAT1 is usually induced in inflammatory disorders, such as corneal neovascularization and sepsis [9, 10]. Thus, we inferred that NEAT1 might be induced in LPS-induced WI-38 cells. As expected, NEAT1 was upregulated under LPS treatment. Further investigation demonstrated that inhibition of NEAT1 significantly improved cell viabilty and suppressed cell apoptosis and inflammation

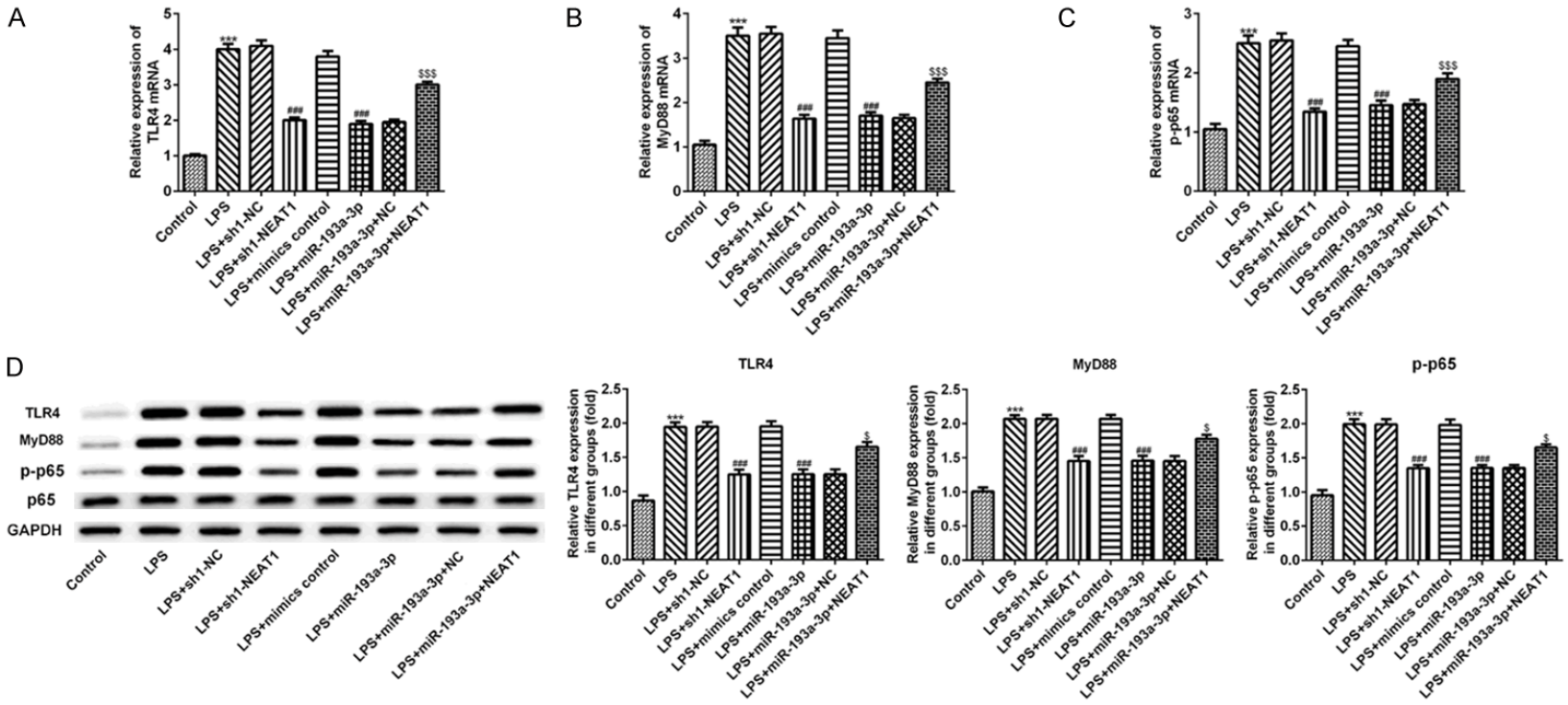


Figure 5. NEAT1/miR-193a-3p regulated TLR4/NF-κB signaling pathway. The effect of NEAT1 and miR-193a-3p on TLR4/NF-κB signaling pathway proteins in LPS-induced WI-38 cells was investigated. mRNA levels and protein expressions of TLR4, myeloid differential protein-88 (MyD88), p-p65 and p-65 were determined by RT-qPCR (A-C) and western blot (D), respectively. Data were expressed as mean ± SD. ***P<0.001 compared with control. ###P<0.001 compared with LPS. $, $$$P<0.05, 0.001 compared with LPS + miR-193a-3p.

that was affected by LPS, suggesting a potential therapetic target for LPS-induced pneumonia. Xiong DD et al pointed out that NEAT1 might exert its role in lung adenocarcinoma by functioning as a ceRNA to regulate miR-193a-3p [16]. Thus we inferred that NEAT1 might also regulate miR-193a-3p to affect LPS-induced inflammatory injury in WI-38 cells. The experimental results verified this inference. Therefore, NEAT1 may exert its role by functioning as a ceRNA to regulate miR-193a-3p in LPS-induced WI-38 cells.

TLR4/NF-κB signaling is implicated in the regulation of inflammatory response in cell proliferation and apoptosis [17]. Generally, TLR4 could specially recognize Gram-negative bacteria, such as LPS. LPS enters the lung and activates TLR4, then activated TLR4 recruits MyD88, leading autophosphorylation of Interleukin-1 receptor-associated kinase (IRAK-1), followed by the activation of NF-κB [18]. NF-κB signaling is a vital cellular signaling involved in immune and inflammatory response [19]. Activated NF-κB results in large production and secretion of pro-inflammatory cytokines through inhibiting apoptosis of macrophages, lymphocytes, neutrophils and other inflammatory cells, deteriorating the inflammatory injury of lung [20]. Consistently, in our study, we found that LPS treatment resulted in an elevated expression of TLR4, MyD88 and p-p65, suggesting an activated NF-κB and inflammatory injury. Inactivating TLR4/NF-κB signaling pathway has been an effective approach and therapeutic target to suppress inflammation. Zhang FX et al disclosed that juglanin could ameliorate LPS-induced neuroinflammation through impeding TLR4/NF-κB [21]. LncRNA MALAT1 inhibition alleviated saturated fatty acid-induced myocardial inflammatory injury through inhibiting the activation of TLR4/NF-κB-mediated inflammatory response [22]. Zhang F et al identified that NEAT1 was involved in TLR4-mediated inflammatory process [23]. Consistently, our study identified that NEAT1 affected the expression of TLR4, MyD88 and p-p65 by regulating miR-193a-3p, indicating that inactivation of the TLR4/NF-κB signaling might be the mechanism for downregulated NEAT1 and overexpressed miR-193a-3p to alleviate LPS-induced inflammatory injury in WI-38 cells.

LPS-induced inflammatory injury in lung is also characterized by epithelial cell apoptosis [24]. Tateda K et al observed acceleration of apoptosis in the infected lungs of mice exposed to hyperoxia [25]. Consistently, our study verified a promoted cell apoptosis in LPS-induced WI-38 cells, as LPS significantly increased cell apoptosis rate, increased the expression of Bax, Cleaved caspase 3/9, and decreased Bcl-2. Bax and Bcl-2 are members of Bcl-2 family that is involved in cell apoptosis. The Bax is regarded as the pre-apoptosis protein and Bcl-2 is the anti-apoptosis protein [26]. Caspase-9, an initiator, is recruited and activated by apoptosome, followed by cleaving procaspase-3 to activate caspase-3. Active caspase-3 degrades multiple cellular proteins and is responsible for morphological changes and DNA fragmentation in cells during apoptosis [27, 28]. Further experiments supported that downregulated NEAT1 decreased cell apoptosis rate and pro-apoptosis-related factors such as Bax, Cleaved caspase-3, and Cleaved caspase-9, and increased anti-apoptosis-related factor such as Bcl-2, thus restoring cell apoptosis induced by LPS. More and more literature illustrated that apoptosis was also attributed to the regulation of TLR4/NF-κB signaling pathway, and TLR4/NF-κB signaling pathway is responsible for inflammatory injury caused by cell apoptosis. Pan YQ et al reported that miR-21 could inhibit the TLR4/NF-κB pathway to reduce myocardial apoptosis and inflammatory factors production [29]. Inhibition of TLR4 effectively alleviated inflammation and apoptosis of high glucose-treated retinal ganglion cells [30]. Besides, NEAT1 was also reported to affect apoptosis and inflammation in sepsis-induced acute kidney by modulating the NF-κB pathway [31]. In our study, both of NEAT1 and miR-193a-3p affected cell apoptosis and inflammatory response induced by LPS by modulating TLR4/NF-κB signaling, and NEAT1 also exert its function by regulating miR-193a-3p. Therefore, we considered that NEAT1 may function as a ceRNA by sponging miR-193a-3p to regulate the activation of TLR4/NF-κB signaling to alleviate inflammation and apoptosis induced by LPS, thus influencing the development of pneumonia.

Conclusion

In conclusion, our study demonstrated NEAT1 was significantly upregulated in LPS-induced WI-38 cells. Knockdown of NEAT1 improved cell viability, suppressed cell apoptosis and inflam-

matory response. Moreover, NEAT1 might exert its role through regulating TLR4/NF-κB signaling pathway by functioning as a ceRNA to regulate miR-193a-3p. Our findings implied that NEAT1 might serve as a neoteric therapy target for pneumonia.

Disclosure of conflict of interest

None.

Address correspondence to: Weixin Nong, Department of Emergency Intensive Care Unit, Guigang City People's Hospital, NO. 1 Zhongshan Road, Gangbei District, Guigang 537100, Guangxi, China. Tel: 0775-4200264; Fax: 0775-4200264; E-mail: nongwx2019@163.com

References

[1] Huang S, Feng C, Chen L, Huang Z, Zhou X, Li B, Wang LL, Chen W, Lv FQ and Li TS. Identification of potential key long non-coding RNAs and target genes associated with pneumonia using long non-coding RNA sequencing (lncRNA-Seq): a preliminary study. Med Sci Monit 2016; 22: 3394-3408.

[2] Ikeda N, Aiba M, Sakurai T, Takahashi M, Yang KS, Tsuda H and Isonuma H. Death in geriatric pneumonia patients. Nihon Ronen Igakkai Zasshi 2011; 48: 282-288.

[3] Dreyfuss D and Ricard JD. Acute lung injury and bacterial infection. Clin Chest Med 2005; 26: 105-112.

[4] Tao Z, Yuan Y and Liao Q. Alleviation of lipopolysaccharides-induced acute lung injury by MiR-454. Cell Physiol Biochem 2016; 38: 65-74.

[5] Zhang H, Zhou D, Ying M, Chen M, Chen P, Chen Z and Zhang F. Expression of long noncoding RNA (lncRNA) small nucleolar RNA host gene 1 (SNHG1) exacerbates hepatocellular carcinoma through suppressing miR-195. Med Sci Monit 2016; 22: 4820-4829.

[6] Zhou Z, Zhu Y, Gao G and Zhang Y. Long noncoding RNA SNHG16 targets miR-146a-5p/CCL5 to regulate LPS-induced WI-38 cell apoptosis and inflammation in acute pneumonia. Life Sci 2019; 228: 189-197.

[7] Feng C, Huang H, Huang S, Zhai YZ, Dong J, Chen L, Huang Z, Zhou X, Li B, Wang LL, Chen W, Lv FQ and Li TS. Identification of potential key genes associated with severe pneumonia using mRNA-seq. Exp Ther Med 2018; 16: 758-766.

[8] Cao J, Zhang Y, Yang J, He S, Li M, Yan S, Chen Y, Qu C and Xu L. NEAT1 regulates pancreatic cancer cell growth, invasion and migration though mircroRNA-335-5p/c-met axis. Am J Cancer Res 2016; 6: 2361-2374.

[9] Bai YH, Lv Y, Wang WQ, Sun GL and Zhang HH. LncRNA NEAT1 promotes inflammatory response and induces corneal neovascularization. J Mol Endocrinol 2018; 61: 231-239.

[10] Chen Y QJ, Chen B, Lin Y, Chen Y, Xie G, Qiu J, Tong H, Jiang D. Long non-coding RNA NEAT1 plays an important role in sepsis-induced acute kidney injury by targeting miR-204 and modulating the NF-κB pathway. Int Immunopharmacol 2018; 59: 252-260.

[11] Liu Z, Yu H and Guo Q. MicroRNA20a promotes inflammation via the nuclear factorkappaB signaling pathway in pediatric pneumonia. Mol Med Rep 2018; 17: 612-617.

[12] Quan B, Zhang H and Xue R. miR-141 alleviates LPS-induced inflammation injury in WI-38 fibroblasts by up-regulation of NOX2. Life Sci 2019; 216: 271-278.

[13] Fan Q, Hu X, Zhang H, Wang S, Zhang H, You C, Zhang CY, Liang H, Chen X and Ba Y. MiR-193a-3p is an important tumour suppressor in lung cancer and directly targets KRAS. Cell Physiol Biochem 2017; 44: 1311-1324.

[14] Liu L, Li Y, Liu S, Duan Q, Chen L, Wu T, Qian H, Yang S and Xin D. Downregulation of miR-193a-3p inhibits cell growth and migration in renal cell carcinoma by targeting PTEN. Tumour Biol 2017; 39: 1010428317711951.

[15] Legnini I, Morlando M, Mangiavacchi A, Fatica A and Bozzoni I. A feedforward regulatory loop between HuR and the long noncoding RNA linc-MD1 controls early phases of myogenesis. Mol Cell 2014; 53: 506-514.

[16] Xiong DD, Li ZY, Liang L, He RQ, Ma FC, Luo DZ, Hu XH and Chen G. The LncRNA NEAT1 accelerates lung adenocarcinoma deterioration and binds to Mir-193a-3p as a competitive endogenous RNA. Cell Physiol Biochem 2018; 48: 905-918.

[17] Xiang NL, Liu J, Liao YJ, Huang YW, Wu Z, Bai ZQ, Lin X and Zhang JH. Abrogating ClC-3 inhibits LPS-induced inflammation via blocking the TLR4/NF-kappaB pathway. Sci Rep 2016; 6: 27583.

[18] Wieland CW, Florquin S, Maris NA, Hoebe K, Beutler B, Takeda K, Akira S and van der Poll T. The MyD88-dependent, but not the MyD88-independent, pathway of TLR4 signaling is important in clearing nontypeable haemophilus influenzae from the mouse lung. J Immunol 2005; 175: 6042-6049.

[19] Zhang H and Sun SC. NF-kappaB in inflammation and renal diseases. Cell Biosci 2015; 5: 63.

[20] Grau A, Tabib A, Grau I, Reiner I and Mevorach D. Apoptotic cells induce NF-kappaB and in-

flammasome negative signaling. PLoS One 2015; 10: e0122440.
[21] Zhang FX and Xu RS. Juglanin ameliorates LPS-induced neuroinflammation in animal models of Parkinson's disease and cell culture via inactivating TLR4/NF-kappaB pathway. Biomed Pharmacother 2018; 97: 1011-1019.
[22] Jia P, Wu N, Jia D and Sun Y. Downregulation of MALAT1 alleviates saturated fatty acid-induced myocardial inflammatory injury via the miR-26a/HMGB1/TLR4/NF-kappaB axis. Diabetes Metab Syndr Obes 2019; 12: 655-665.
[23] Zhang F, Wu L, Qian J, Qu B, Xia S, La T, Wu Y, Ma J, Zeng J, Guo Q, Cui Y, Yang W, Huang J, Zhu W, Yao Y, Shen N and Tang Y. Identification of the long noncoding RNA NEAT1 as a novel inflammatory regulator acting through MAPK pathway in human lupus. J Autoimmun 2016; 75: 96-104.
[24] Chopra M, Reuben JS and Sharma AC. Acute lung injury:apoptosis and signaling mechanisms. Exp Biol Med (Maywood) 2009; 234: 361-371.
[25] Tateda K, Deng JC, Moore TA, Newstead MW, Paine R 3rd, Kobayashi N, Yamaguchi K and Standiford TJ. Hyperoxia mediates acute lung injury and increased lethality in murine Legionella pneumonia: the role of apoptosis. J Immunol 2003; 170: 4209-4216.
[26] Banasiak KJ, Xia Y and Haddad GG. Mechanisms underlying hypoxia-induced neuronal apoptosis. Prog Neurobiol 2000; 62: 215-249.
[27] McIlwain DR, Berger T and Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol 2013; 5: a008656.
[28] Tamura R, Takada M, Sakaue M, Yoshida A, Ohi S, Hirano K, Hayakawa T, Hirohashi N, Yura K and Chiba K. Starfish Apaf-1 activates effector caspase-3/9 upon apoptosis of aged eggs. Sci Rep 2018; 8: 1611.
[29] Pan YQ, Li J, Li XW, Li YC, Li J and Lin JF. Effect of miR-21/TLR4/NF-kappaB pathway on myocardial apoptosis in rats with myocardial ischemia-reperfusion. Eur Rev Med Pharmacol Sci 2018; 22: 7928-7937.
[30] Hu L, Yang H, Ai M and Jiang S. Inhibition of TLR4 alleviates the inflammation and apoptosis of retinal ganglion cells in high glucose. Graefes Arch Clin Exp Ophthalmol 2017; 255: 2199-2210.
[31] Chen Y, Qiu J, Chen B, Lin Y, Chen Y, Xie G, Qiu J, Tong H and Jiang D. Long non-coding RNA NEAT1 plays an important role in sepsis-induced acute kidney injury by targeting miR-204 and modulating the NF-kappaB pathway. Int Immunopharmacol 2018; 59: 252-260.