

Received: 2019.10.24

Accepted: 2020.01.17

Available online: 2020.02.22

Published: 2020.04.22

# Long Non-Coding RNA (lncRNA) CRNDE Regulated Lipopolysaccharides (LPS)-Induced MRC-5 Inflammation Injury Through Targeting MiR-141

**Authors' Contribution:**

Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

**ABC 1 Xingping Tang\***  
**CD 1 Ting Wang\***  
**CD 1 Caihong Qiu**  
**CG 2 Fangfang Zheng**  
**AE 3 Jiao Xu**  
**AD 4 Beilong Zhong**

1 Department of Respiratory Medicine, Yidu Central Hospital of Weifang, Qingzhou, Shandong, P.R. China  
2 Department of Pediatrics, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong, P.R. China  
3 Department of Respiratory Medicine, Affiliated Wujin Hospital of Jiangsu University, Wujin Clinical College of Xuzhou Medical University, Changzhou, Jiangsu, P.R. China  
4 Department of Thoracic Surgery, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong, P.R. China

\* Xingping Tang and Ting Wang contributed equally to this work

**Corresponding Authors:** Jiao Xu, e-mail: ngsxd@163.com, Beilong Zhong, e-mail: taishu1967215@126.com  
**Source of support:** Departmental sources

**Background:** Pneumonia is a common disease with high morbidity and even death. In our country, pneumonia is the leading cause of child death. Therefore, research on the pathogenesis of pneumonia can help improve the treatment of pneumonia. Long non-coding RNA (lncRNA) is an important regulator of disease development, and its regulatory mechanism is closely related to cellular processes. However, the function and regulatory network of lncRNA is not fully elucidated in pneumonia.


**Material/Methods:** Quantitative real-time polymerase chain reaction (qRT-PCR) was applied to detect the expression of CRNDE and miR-141 in lipopolysaccharides (LPS)-induced MRC-5 cells and pneumonia tissues. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-tetrazolium) assay was used to assess cell proliferation. Flow cytometry assay was performed to detect cell apoptosis in LPS-induced MRC-5 cells. Enzyme-linked immunosorbent assay and western blot were used to measure the levels of interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor (TNF)- $\alpha$ , respectively. In addition, luciferase reporter assay and RNA immunoprecipitation (RIP) assay were applied to prove the relationship between CRNDE and miR-141.

**Results:** In this study, we found that CRNDE expression was induced in LPS-induced MRC-5 cells and pneumonia tissues. Moreover, miR-141 expression was low in LPS-induced MRC-5 cells and was verified as a target miRNA of CRNDE by using luciferase reporter assay and RIP assay. The downregulation of CRNDE and upregulation of miR-141 promoted cell viability, inhibited cell apoptosis, as well as decreased the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$ . Moreover, we demonstrated that si-CRNDE transfection increased cell viability and suppressed cell apoptosis and the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , which were alleviated by anti-miR-141 transfection in LPS-induced MRC-5 cells.

**Conclusions:** In this study, we found that downregulation of CRNDE and upregulation of miR-141 inhibited cell apoptosis and inflammation response and promoted cell viability in LPS-induced MRC-5 cells. Low CRNDE expression increased cell growth and suppressed inflammation response, which was impaired by inhibition of miR-141. These results suggested that a novel therapeutic target was found in pneumonia treatment.

**MeSH Keywords:** Inflammation Mediators • MicroRNAs • Pneumonia, Aspiration

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/920928>

 2558

 —

 6

 48



## Background

Pneumonia in children is mainly caused by inflammation of the lungs caused by bacteria and viruses; it is one of the main causes of infant and child death [1–3]. Due to children with pneumonia have fewer symptoms and higher infection rates, it is urgent to find effective diagnosis and treatment strategies. Interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor (TNF)- $\alpha$  are very important pro-inflammatory factors that release apoptosis and necrosis, accumulate leukocytes, and inhibit microvascular function [2,4–6]. Regulatory factors related to the inflammatory response to pneumonia have important implications for the diagnosis and treatment of pneumonia.

Long non-coding RNA (lncRNA) is a type of non-coding RNA of more than 200 nucleotides in length [7]. As an important regulator, lncRNA is involved in the physiological and pathological processes of cells and tissues [8,9]. Differentially expressed lncRNA is closely related to cell proliferation, apoptosis, migration, invasion, drug resistance, and inflammatory and immune response in different diseases, such as cancer and pneumonia [10–14]. In pneumonia, the regulation mechanism of lncRNA plays an indispensable role; for example, lncRNA HAGLROS was shown to be involved in cell autophagy and apoptosis of pneumonia through regulating the miR-100/NF- $\kappa$ B axis [15]. CRNDE is also related to the production and development of various diseases and is highly expressed in renal cell carcinoma, cervical cancer, and bladder cancer [16–19]. Similarly, the expression of CRNDE in pneumonia increases significantly, indicating that CRNDE is also involved in some physiological and pathological processes of pneumonia, including inflammatory response and cell growth [20]. However, the function and regulatory network of CRNDE has not been fully elucidated in pneumonia.

In this study, we used lipopolysaccharides (LPS) to induce MCR-5 cell line to construct a pneumonia model, mainly to study the regulatory function and regulatory network of CRNDE in pneumonia, aiming to analyze the important role of CRNDE regulation mechanism in pneumonia cell progression and inflammatory response.

## Material and Methods

### Patients and sample collection

This experiment was approved by the Ethics Committee of The Fifth Affiliated Hospital of Sun Yat-sen University. A total of 30 children (17 males and 13 females; mean age $\pm$ standard deviation, 9.8 $\pm$ 3.25 years) with pneumonia were recruited to the present study. We exclude patients with other complications or patients who had received anti-inflammatory treatment. In addition, 15 gender-matched children (7 males and 8

females; mean age $\pm$ standard deviation, 10.6 $\pm$ 2.54 years) with fever were included as the control group. All children were from The Fifth Affiliated Hospital of Sun Yat-sen University. Their serums were collected, then centrifuged and stored in  $-80^{\circ}\text{C}$ . Informed consent was provided by all patients and/or their guardians.

### Cell culture and LPS treatment

Normal human embryo lung cell line MRC-5 was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and incubated in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St Louis, MO, USA) containing 10% fetal bovine serum, 1% penicillin/streptomycin (Invitrogen), and then maintained in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C. Different concentrations of LPS (5, 10, and 20  $\mu\text{g}/\text{mL}$ , Sigma, purified by phenol extraction and gel filtration) was added into medium for 12 hours to construct pneumonia model.

### Cell transfection

The LPS-induced MRC-5 cells were seeded into 6-well plates and incubated in DMEM overnight. Si-CRNDE, pc-CRNDE (CRNDE), miR-141, anti-miR-141 and their negative controls (si-NC, pcDNA, miR-NC, anti-miR-NC) were purchased from Ribobio (Guangzhou, China). All plasmid and oligos were transfected into LPS-induced MRC-5 cells using Lipofectamine 2000 Reagent (Life Technologies Corporation, Grand Island, NY, USA).

### Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from serum and cells using TRIzol reagent (Invitrogen). cDNA of miR-141 was reverse transcribed with TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). cDNA of CRNDE was reverse transcribed with Reverse Transcription Reagents (Applied Biosystems). U6 and glyceraldehyde phosphate dehydrogenase (GAPDH) were normalized against an internal control for miR-141 and CRNDE, respectively. Real-time polymerase chain reaction (RT-PCR) was performed using SYBR Green Real-time PCR analysis (Takara) according to the manufacturer's protocol. The 2<sup>- $\Delta\Delta$ Ct</sup> method was applied to calculate the relative expression of miR-141 and CRNDE.

The primer sequences:

lncRNA CRNDE, forward, 5'-TGAAGGAAGGAAGTGGTGCA-3' and reverse, 5'-TCCAGTGGCATCTACAAGA-3';  
GAPDH, forward, 5'-TGTTCTCATGGGTGTAAC-3' and reverse, 5'-ATGGCATGGACTGTGGTCAT-3';  
miR-141, forward, 5'-AAGACGTACTCAGGCCATGTCC-3' and reverse 5'-GACCCAAATGTCGACAGTCC-3';  
U6, forward 5'-CTCGCTTCGGCAGCAC-3' and reverse 5'-AACGCTTACGAATTTGCGT-3'.

## Western blot

Transfected cells were washed with phosphate-buffered saline (PBS) and extracted by re-suspending cell pellets in RNA immunoprecipitation assay (RIPA) buffer (Beyotime, Haimen, China). The proteins were separated using 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Inc.). The membranes were incubated with the primary antibodies against IL-1 $\beta$ , IL-6, TNF- $\alpha$  and GAPDH (1: 2000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. Subsequently, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1: 2000 dilution, Santa Cruz Biotechnology) for 1 hour. The blot was detected by using an ECL Plus Western Blotting Substrate (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

## MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-tetrazolium bromide) assay

Cell viability was measured using MTT assay. Briefly,  $2 \times 10^3$  LPS-induced MRC-5 cells were seeded into a 96-well plate and incubated overnight. Then 10  $\mu$ L MTT solution (Sigma-Aldrich, St. Louis, MO, USA) was added into plates and cultured for 4 hours at room temperature. We removed the supernatant after centrifugation and added 150  $\mu$ L of dimethyl sulfoxide. The optical density (OD) was measured with a microplate reader (Bio-Rad, Hercules, CA, USA) at a wavelength of 450 nm.

## Flow cytometry

Annexin V FITC/propidium iodide (PI) staining assay (Invitrogen, CA, USA) was applied to detect cell apoptosis according to the manufacturer's instruction. Cells were harvested and washed in PBS. Next, each plate was stained with FITC-Annexin V and PI (BD Biosciences, San Jose, CA, USA) for 40 minutes. Flow cytometry (BD Biosciences) was used to detect cell apoptosis.

## Luciferase reporter assay

The 3'-UTR of lncRNA CRNDE (containing miR-141 binding sites) or the mutation of CRNDE (containing mutation sites) was synthesized using PCR and inserted into pMIR-REPORT™ (ThermoFisher Scientific) to construct CRNDE wild-type reporter vector (CRNDE-WT) or CRNDE mutation reporter vector (CRNDE-MUT). CRNDE-WT or CRNDE-MUT and miR-NC or miR-141 were transfected into MRC-5 cells, respectively. After transfection for 48 hours, cells were harvested for measuring the luciferase activity using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instruction.

## RNA immunoprecipitation assay and enzyme-linked immunosorbent assay (ELISA)

Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) was used to measure the enrichment of CRNDE in cells. Transfected cells were incubated with the Ago2 antibody (Abcam, Cambridge, MA, USA). Normal mouse IgG and miR-NC transfection act as negative and positive controls, respectively.

Cell collected and the inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) were measured by using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

## Statistical analysis

All data were presented as mean  $\pm$  standard deviation (SD) and all experiments were repeated at least 3 times. The data were analyzed and displayed using GraphPad Prism 7.0. Student's *t*-test or one-way ANOVA were used to compare between groups. \* *P* < 0.05 was considered as statistically significant.

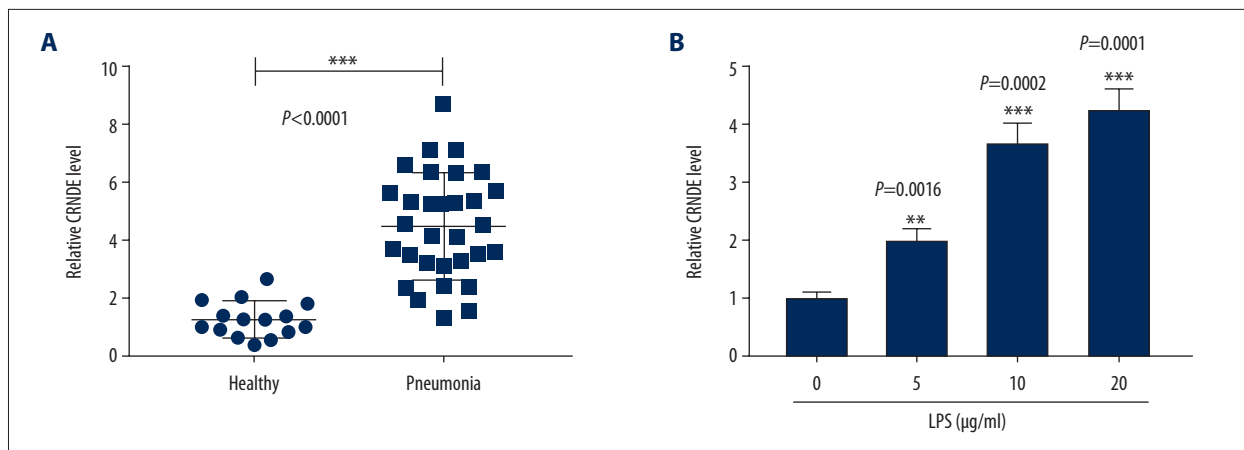
## Results

### lncRNA CRNDE was induced in pneumonia and LPS-induced MRC-5 cells

To further explore the expression of CRNDE in pneumonia, 15 healthy samples and 30 pneumonia samples were collected. qRT-PCR determined that the expression of CRNDE in pneumonia was higher than healthy controls (Figure 1A). Otherwise, LPS was used to construct the pediatric pneumonia model. Different concentrations of LPS (5, 10, and 20  $\mu$ g/mL) were used to induced MRC-5 cells. The results showed that with the increase of LPS concentration, CRNDE expression gradually increased, especially in the 10  $\mu$ g/mL and 20  $\mu$ g/mL of LPS (*P* < 0.01) (Figure 1B). We selected 10  $\mu$ g/mL of LPS to use in further experiments. Thus, lncRNA CRNDE was induced in pneumonia and LPS-induced MRC-5 cells.

### Si-CRNDE promoted cell growth and inhibited inflammation response in LPS-induced MRC-5 cells

To further explore the function of CRNDE in pneumonia, si-CRNDE was transfected into LPS-induced MRC-5 cells, which obviously inhibited CRNDE expression (Figure 2A). As shown in Figure 2B–2I, we found that LPS suppressed cell viability, induced cell apoptosis, and promoted the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in MRC-5 cells. However, cell apoptosis in LPS+si-CRNDE group was higher than that in LPS+si-NC group. Moreover, si-CRNDE transfection inhibited cell apoptosis



**Figure 1.** LncRNA CRNDE was induced in pneumonia and LPS-induced MRC-5 cells. (A) The expression of CRNDE was detected in pneumonia by qRT-PCR. (B) The expression of CRNDE was detected in different concentrations of LPS (5, 10, 20 µg/mL) by qRT-PCR. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ . lncRNA – long non-coding RNA; LPS – lipopolysaccharide; qRT-PCR – quantitative real-time polymerase chain reaction.

and reduced the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in LPS-induced MRC-5 cells. These results showed that si-CRNDE could alleviate the effects of LPS on MRC-5 cells. Thus, si-CRNDE promoted cell growth and inhibited inflammation response in LPS-induced MRC-5 cells, implying that downregulation of CRNDE could alleviate cell inflammation and injury in pneumonia.

### miR-141 was a target of CRNDE

To further investigate the regulatory network of CRNDE, miR-141 was predicted was a potential target of CRNDE using DIANA Tool (Figure 3A). To ensure the relationship between CRNDE and miR-141, CRNDE-WT, and CRNDE-MUT were transfected into miR-141 or miR-NC transfection MRC-5 cell. The results of the luciferase reporter assay showed that luciferase activity was significantly reduced when miR-141 was bound to CRNDE-WT, but not CRNDE-MUT, indicating that miR-141 was a target of CRNDE (Figure 3B). RIP assay was used to further verify the direct binding between miR-141 and CRNDE. As shown in Figure 3C, compared with the miR-NC group, the expression of CRNDE was obviously enriched in the miR-141 groups. In addition, CRNDE transfection inhibited miR-141 expression while si-CRNDE induced miR-141 expression in MRC-5 cells (Figure 3D). These results indicated that miR-141 was a target of CRNDE and was regulated by CRNDE.

### miR-141 was decreased in pneumonia and LPS-induced MRC-5 cells

To analyze the expression of miR-141 in pneumonia and LPS-induced MRC-5 cells, qRT-PCR was applied to detect its expression. As shown in Figure 4A, miR-141 expression was significantly inhibited in pneumonia. Additionally, with the increase of LPS, the expression of miR-141 was gradually decreased in

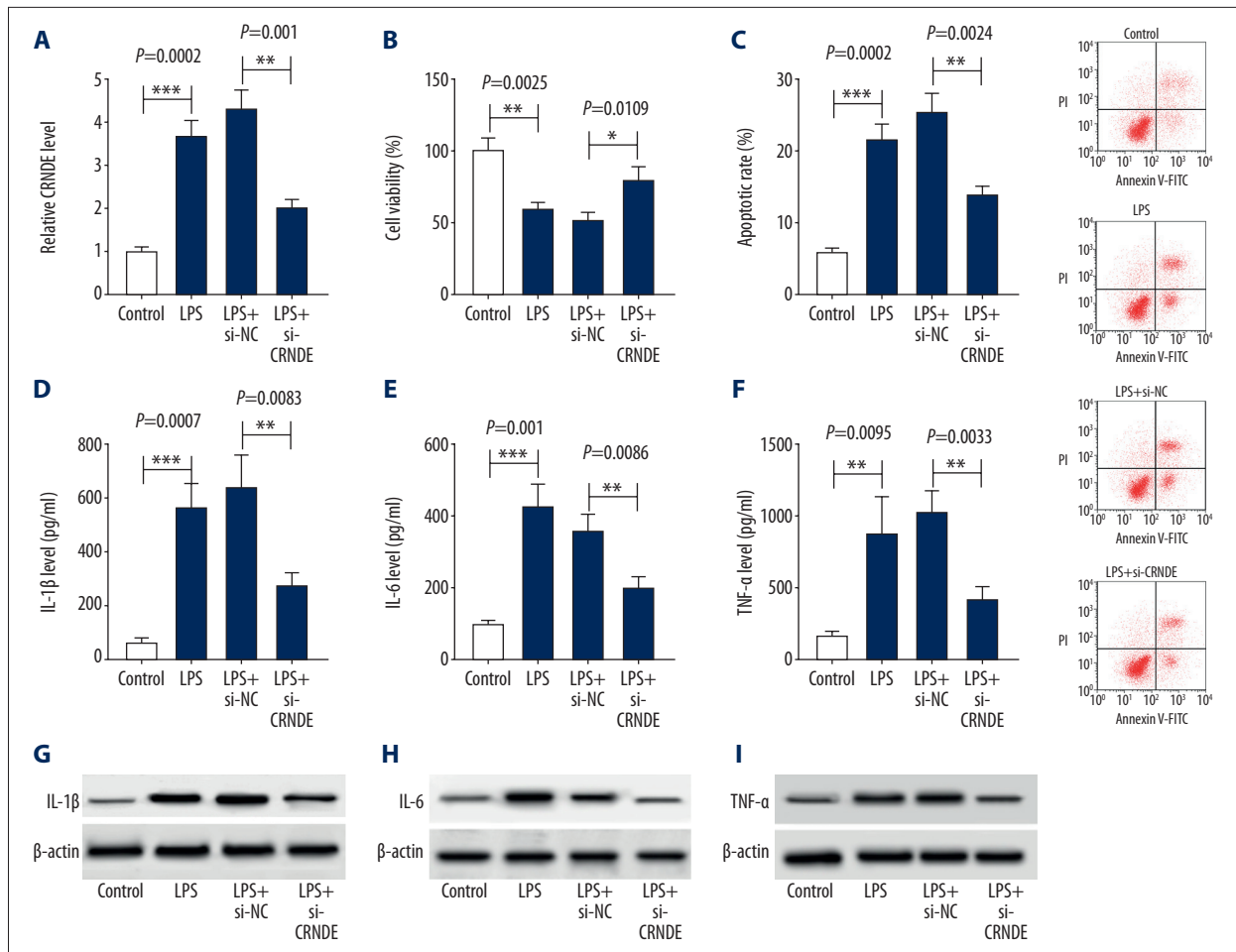
MRC-5 cells, suggesting that LPS inhibited miR-141 expression in MRC-5 cells (Figure 4B).

### Overexpression of miR-141 contributed to cell growth and suppressed inflammation response in LPS-induced MRC-5 cells

The function of miR-141 in pneumonia was investigated by transfecting miR-141 into the LPS-induced MRC-5 cells, which expressed high levels of miR-141 (Figure 5A). As shown in Figure 5B and 5C, miR-141 transfection promoted cell viability and inhibited cell apoptosis in LPS-induced MRC-5 cells. Otherwise, the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were measured using ELISA and western blot. The results showed that overexpression of miR-141 decreased the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  (Figure 5D–5I). Briefly, overexpression of miR-141 contributed to cell growth and suppressed inflammation response in LPS-induced MRC-5 cells.

### Downregulation of CRNDE promoted cell growth and inhibited inflammation response in LPS-induced MRC-5 cells, which impaired by inhibition of miR-141

Rescue experiments were applied to determine the regulatory network of CRNDE and miR-141 in LPS-induced MRC-5 cells. As shown in Figure 6, compared with the si-NC group, the expression of miR-141 and cell viability were remarkably induced, while cell apoptosis and the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were significantly reduced in LPS-induced MRC-5 cells. However, the expression of miR-141 and cell viability were lower in the si-CRNDE+anti-miR-141 group than the si-CRNDE+anti-miR-NC group. Cell apoptosis and the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were higher in the si-CRNDE+anti-miR-141 group than the si-CRNDE+anti-miR-NC group. These results determined



**Figure 2.** Si-CRNDE promoted cell growth and inhibited inflammation response in LPS-induced MRC-5 cells. (A) The expression of CRNDE was measured in control, LPS, LPS+si-NC, and LPS+si-CRNDE groups by qRT-PCR. (B) Cell viability was detected in control, LPS, LPS+si-NC, and LPS+si-CRNDE groups by MTT assay. (C) Cell apoptosis was detected in control, LPS, LPS+si-NC, and LPS+si-CRNDE groups by flow cytometry. (D–F) The levels of IL-1β (D), IL-6 (E), and TNF-α (F) were measured in control, LPS, LPS+si-NC, and LPS+si-CRNDE groups by ELISA assay. (G–I) The levels of IL-1β (G), IL-6 (H), and TNF-α (I) were detected in control, LPS, LPS+si-NC, and LPS+si-CRNDE groups with western blot assay. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ . LPS – lipopolysaccharide; qRT-PCR – quantitative real-time polymerase chain reaction; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-tetrazolium; IL – interleukin; TNF – tumor necrosis factor; ELISA – enzyme-linked immunosorbent assay.

that anti-miR-141 could alleviate the effect of si-CRNDE on LPS-induced MRC-5 cells and also indicated that CRNDE affected cell growth and inflammation response in LPS-induced MRC-5 cells by targeting miR-141.

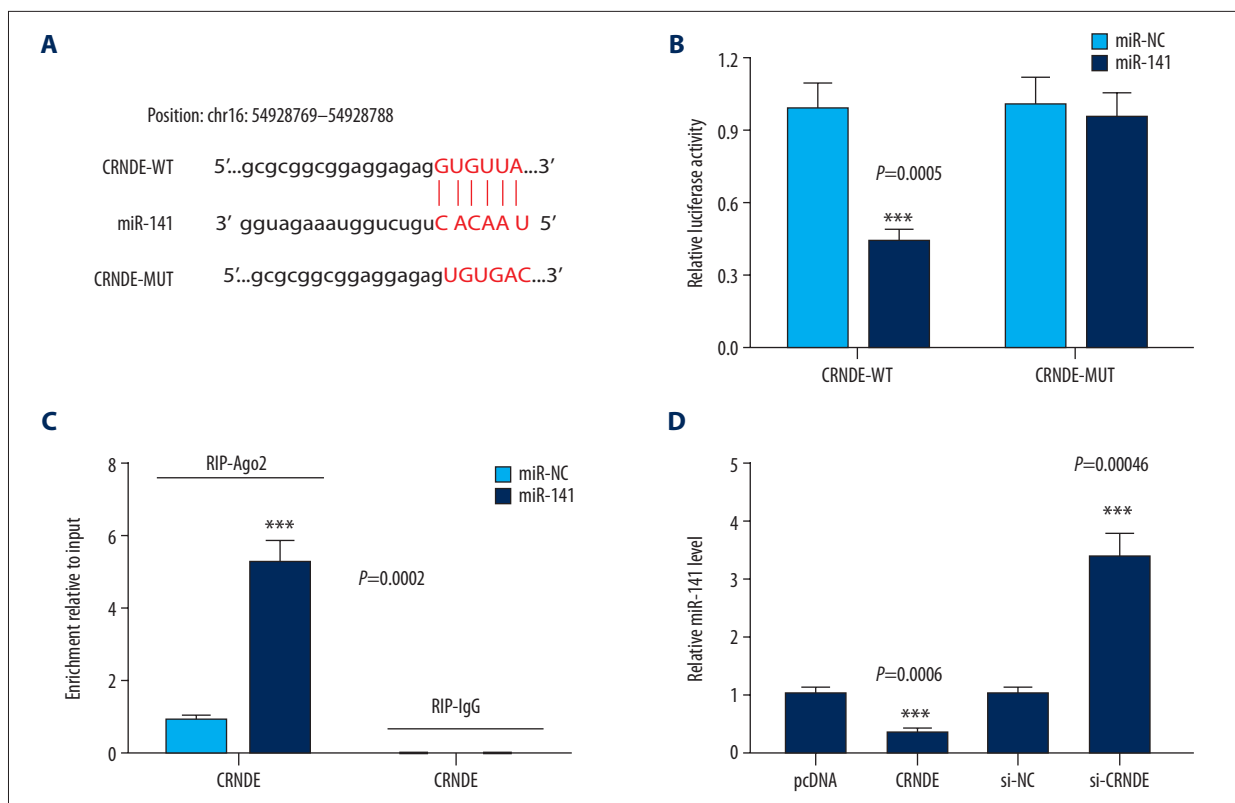
## Discussion

In this study, we found that the CRNDE/miR-141 axis has potential regulatory functions in pediatric pneumonia. To demonstrate the effects of CRNDE and miR-141 in pneumonia, we induced MRC-5 cells to construct a pneumonia model using LPS; we then studied their function in pneumonia by reducing the expression of CRNDE and increasing the expression of

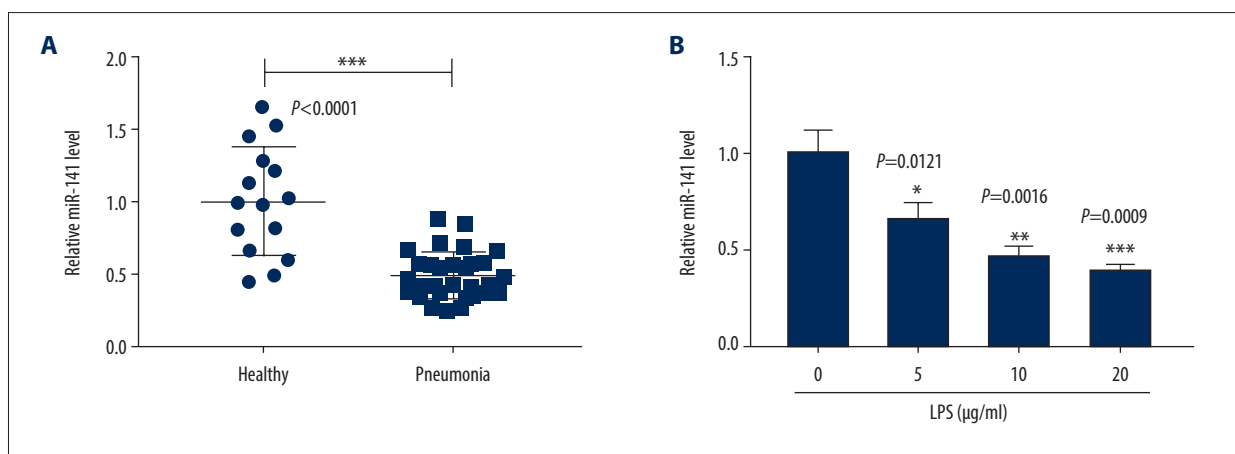
miR-141. We found that the effects of lowering CRNDE and increasing miR-141 expression in pneumonia were similar, both promoting cell growth and alleviating inflammatory responses. Moreover, both luciferase reporter assay and RIP assay demonstrated that miR-141 was a target miRNA of CRNDE. Furthermore, the rescue experiments also demonstrated that CRNDE affects cell growth and inflammation in pneumonia by regulating miR-141. Our results show that the CRNDE/miR-141 axis is involved in the development and inflammatory response of pneumonia.

Recently, many studies have shown that lncRNA plays an important role in disease production, and physiological and biochemical mechanisms. It has been reported that many differentially

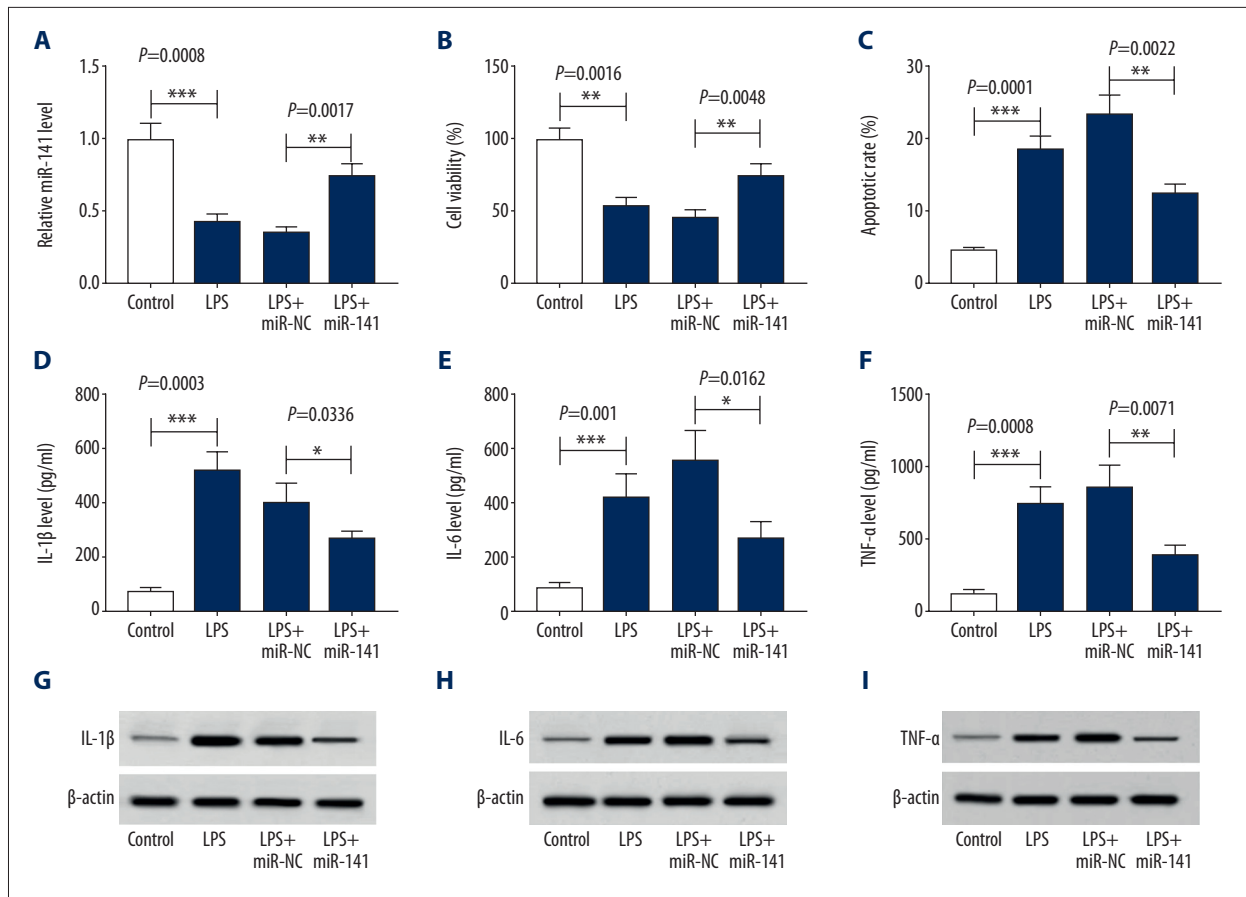




**Figure 3.** MiR-141 was a target of CRNDE. **(A)** Putative miR-141 binding sequence and mutation sequence of CRNDE. **(B)** Luciferase reporter assay was used to verify that miR-141 was a target of CRNDE. **(C)** RIP assay was applied to ensure CRNDE could bind to miR-141. **(D)** The expression of miR-141 was detected in pcDNA, CRNDE, si-NC, and si-CRNDE groups by qRT-PCR. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ . RIP assay – RNA immunoprecipitation; qRT-PCR – quantitative real-time polymerase chain reaction.



**Figure 4.** MiR-141 was decreased in pneumonia and LPS-induced MRC-5 cells. **(A)** The expression of miR-141 was detected in pneumonia by qRT-PCR. **(B)** The expression of miR-141 was detected in different concentration LPS (5, 10, 20 µg/mL) by qRT-PCR. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ . LPS – lipopolysaccharide; qRT-PCR – quantitative real-time polymerase chain reaction.

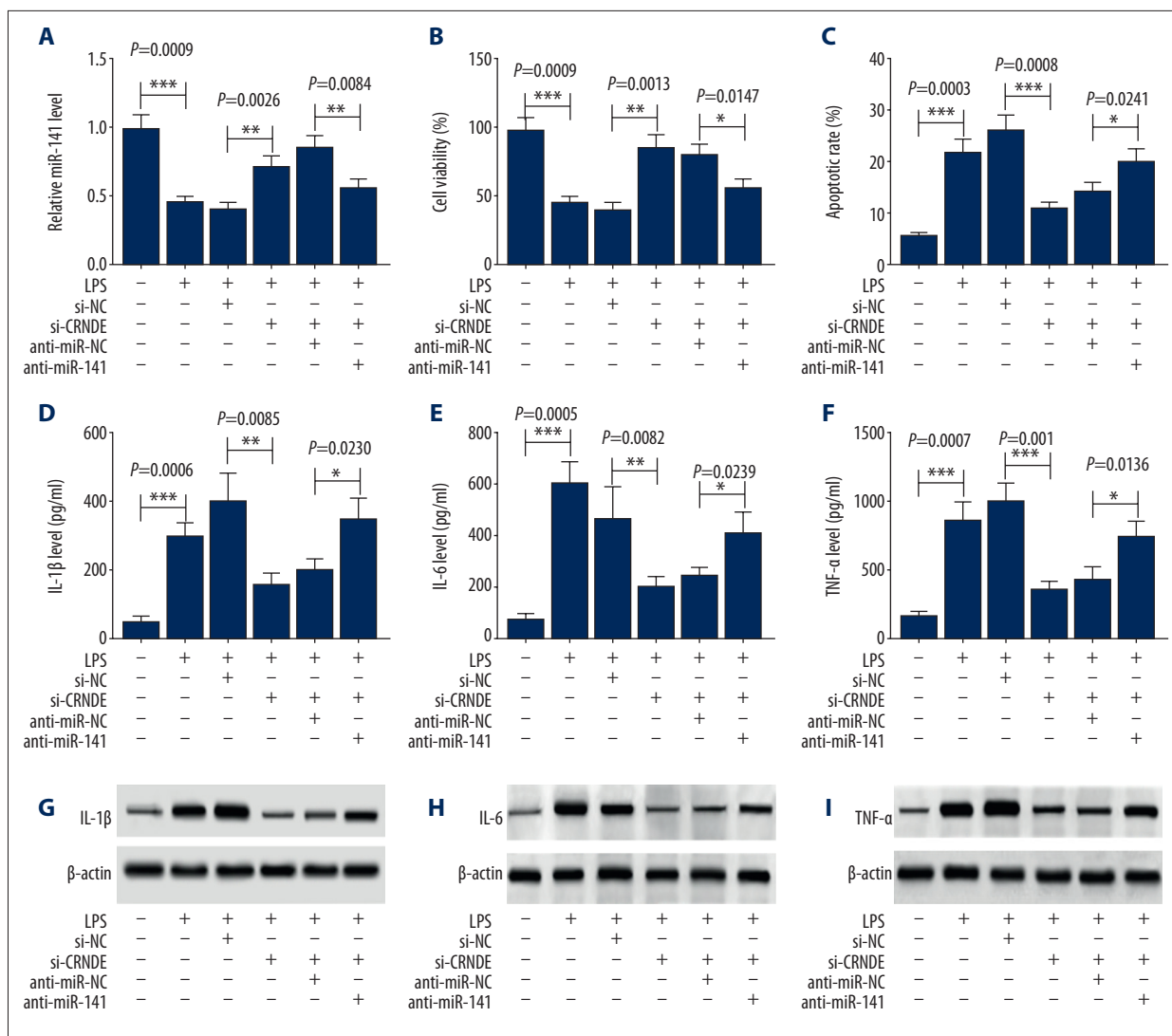


**Figure 5.** Overexpression of miR-141 contributed to cell growth and suppressed inflammation response in LPS-induced MRC-5 cells. (A) The expression of miR-141 was measured in control, LPS, LPS+miR-NC, and LPS+miR-141 groups by qRT-PCR. (B) Cell viability was detected in control, LPS, LPS+miR-NC, and LPS+miR-141 groups by MTT assay. (C) Cell apoptosis was detected in control, LPS, LPS+miR-NC, and LPS+miR-141 groups by flow cytometry. (D–F) The levels of IL-1β (D), IL-6 (E), and TNF-α (F) were measured in control, LPS, LPS+miR-NC, and LPS+miR-141 groups by ELISA assay. (G–I) The levels of IL-1β (G), IL-6 (H), and TNF-α (I) were detected in control, LPS, LPS+miR-NC, and LPS+miR-141 groups with western blot assay. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ . LPS – lipopolysaccharide; qRT-PCR – quantitative real-time polymerase chain reaction; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-tetrazolium; IL – interleukin; TNF – tumor necrosis factor; NC – negative control; ELISA – enzyme-linked immunosorbent assay.

expressed lncRNAs are involved in biological metabolism and tissue transfer of cells, especially in cancers [21–24]. For example, lncRNA MALAT1 is involved in the cellular processes of colon cancer, including apoptosis, migration, proliferation and invasion [25–28]. More than that, lncRNA could be used as a biomarker for the diagnosis and treatment of diseases [29,30]. CRNDE has been found to be highly expressed in a variety of cancers and is involved in cell proliferation, invasion, metastasis, apoptosis and inflammatory responses [19,31–34]. Li et al. determined that CRNDE can activate the TLR3/NF- $\kappa$ B/cytokine signaling pathway to regulate inflammatory response in glioma cells [32]. Consistent with previous studies [20], we found that CRNDE was highly expressed in pneumonia cells, which significantly promoted cell proliferation, inhibited apoptosis, and reduced the release of pro-inflammatory factors.

Generally, lncRNA targets miRNA formation regulatory networks were involved in various physiological and biochemical processes of cells in diseases [35–38]. Therefore, miR-141 was verified to be a target miRNA for CRNDE in this experiment. In addition, miR-141 is also involved in cellular processes in many diseases, including cancers and pneumonia [39–42]. The study of Wang et al. have shown that miR-141, as a tumor suppressor, participated in glioma growth through targeting ATF5 [43]. In this experiment, miR-141 expression was low in pneumonia, and the effects of high miR-141 expression on cells were consistent with the effect of reducing CRNDE expression on cells, indicating that CRNDE negatively regulates miR-141 in pneumonia.

When pneumonia is produced, inflammatory factors are released, which could promote apoptosis and inhibit cell



**Figure 6.** Downregulation of CRNDE promoted cell growth and inhibited inflammation response in LPS-induced MRC-5 cells, which were impaired by inhibition of miR-141. (A) The expression of miR-141 was measured in control, LPS, LPS+si-NC, LPS+si-CRNDE, LPS+si-CRNDE+anti-miR-NC, and LPS+si-CRNDE+anti-miR-141 groups by qRT-PCR. (B) Cell viability was detected in control, LPS, LPS+si-NC, LPS+si-CRNDE, LPS+si-CRNDE+anti-miR-NC, and LPS+si-CRNDE+anti-miR-141 groups by MTT assay. (C) Cell apoptosis was detected in control, LPS, LPS+si-NC, LPS+si-CRNDE, LPS+si-CRNDE+anti-miR-NC, and LPS+si-CRNDE+anti-miR-141 groups by flow cytometry. (D–F) The levels of IL-1β (D), IL-6 (E), and TNF-α (F) were measured in control, LPS, LPS+si-NC, LPS+si-CRNDE, LPS+si-CRNDE+anti-miR-NC, and LPS+si-CRNDE+anti-miR-141 groups by ELISA assay. (G–I) The levels of IL-1β (G), IL-6 (H), and TNF-α (I) were detected in control, LPS, LPS+si-NC, LPS+si-CRNDE, LPS+si-CRNDE+anti-miR-NC, and LPS+si-CRNDE+anti-miR-141 groups with western blot assay. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ . LPS – lipopolysaccharide; qRT-PCR – quantitative real-time polymerase chain reaction; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-tetrazolium; IL – interleukin; TNF – tumor necrosis factor; ELISA – enzyme-linked immunosorbent assay.

proliferation [44,45]. Relief of the inflammatory response contributes to pneumonia treatment. In this study we found that CRNDE-reduced expression and miR-141-enhanced expression could suppress inflammatory factors, suggesting CRNDE and miR-141 also play an important role in the treatment of pneumonia.

Notably, in this study we demonstrated for the first time that miR-141 was a target miRNA of CRNDE, which could further improve the regulatory network of CRNDE in pneumonia, providing a new target site for the treatment of pneumonia, and consolidating the important role of CRNDE in the diagnosis of pneumonia. In addition, accumulating evidence indicates that miR-141 can regulate the physiological mechanisms by which



downstream target genes affect cells [46–48]. For example, in hepatocellular carcinoma cells and ovarian cancer cells, the miR-141/KEAP1 axis was shown to be involved in the mechanism of cell growth and chemoresistance [46,48]. Therefore, we speculate that CRNDE affected miR-141 expression and further influenced downstream target genes of miR-141 to regulating the biochemical mechanisms of cells, which will be the focus of our next research.

## References:

1. Prina E, Ranzani OT, Torres A: Community-acquired pneumonia. *Lancet*, 2015; 386: 1097–108
2. Schlager R, Queen K, Simmon KE et al: Viral pathogen detection by metagenomics and pan-viral group polymerase chain reaction in children with pneumonia lacking identifiable etiology. *J Infect Dis*, 2017; 215: 1407–15
3. Stadler JAM, Andronikou S, Zar HJ: Lung ultrasound for the diagnosis of community-acquired pneumonia in children. *Pediatr Radiol*, 2017; 47: 1412–19
4. Zhang J, An J: Cytokines, inflammation, and pain. *Int Anesthesiol Clin*, 2007; 45: 27–37
5. Bessler H, Djaldetti R, Salman H et al: IL-1 $\beta$ , IL-2, IL-6 and TNF- $\alpha$  production by peripheral blood mononuclear cells from patients with Parkinson's disease. *Biomed Pharmacother*, 1999; 53: 141–45
6. Wu X, Lu Y, Dong Y et al: The inhalation anesthetic isoflurane increases levels of proinflammatory TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . *Neurobiol Aging*, 2012; 33: 1364–78
7. Rinn JL: LncRNAs: Linking RNA to chromatin. *Cold Spring Harb Perspect Biol*, 2014; 6(8). pii: a018614
8. Han P, Li W, Lin CH et al: A long noncoding RNA protects the heart from pathological hypertrophy. *Nature*, 2014; 514: 102–6
9. Li L, Chang HY: Physiological roles of long noncoding RNAs: Insight from knockout mice. *Trends Cell Biol*, 2014; 24: 594–602
10. Chi X, Ding B, Zhang L et al: LncRNA GAS5 promotes M1 macrophage polarization via miR-455-5p/SOCS3 pathway in childhood pneumonia. *J Cell Physiol*, 2019; 234: 13242–51
11. Fang Q, Chen XY, Zhi XT: Long non-coding RNA (LncRNA) urothelial carcinoma associated 1 (UCA1) increases multi-drug resistance of gastric cancer via downregulating miR-27b. *Med Sci Monit*, 2016; 22: 3506–13
12. Li H, Yu B, Li J et al: Overexpression of lncRNA H19 enhances carcinogenesis and metastasis of gastric cancer. *Oncotarget*, 2014; 5: 2318–29
13. Nie W, Ge HJ, Yang XQ et al: LncRNA-UCA1 exerts oncogenic functions in non-small cell lung cancer by targeting miR-193a-3p. *Cancer Lett*, 2016; 371: 99–106
14. Meng J, Chen Y, Zhang C: Protective impacts of long noncoding RNA taurine-upregulated 1 against lipopolysaccharide-evoked injury in MRC-5 cells through inhibition of microRNA-127. *J Cell Biochem*, 2019; 120(9): 14928–35
15. Liu M, Han T, Shi S et al: Long noncoding RNA HAGLROS regulates cell apoptosis and autophagy in lipopolysaccharides-induced WI-38 cells via modulating miR-100/NF- $\kappa$ B axis. *Biochem Biophys Res Commun*, 2018; 500: 589–96
16. Shao K, Shi T, Yang Y et al: Highly expressed lncRNA CRNDE promotes cell proliferation through Wnt/ $\beta$ -catenin signaling in renal cell carcinoma. *Tumor Biol*, 2016; 37: 15997–6004
17. Kiang KMY, Zhang X, Zhang GP et al: CRNDE expression positively correlates with EGFR activation and modulates glioma cell growth. *Target Oncol*, 2017; 12: 353–63
18. Cheng J, Chen J, Zhang X et al: Overexpression of CRNDE promotes the progression of bladder cancer. *Biomed Pharmacother*, 2018; 99: 638–44
19. Ma X, Zhang W, Zhang R et al: Overexpressed long noncoding RNA CRNDE with distinct alternatively spliced isoforms in multiple cancers. *Front Med*, 2019; 13(3): 330–43
20. Zhu-Ge D, Yang YP, Jiang ZJ: Knockdown CRNDE alleviates LPS-induced inflammation injury via FOXM1 in WI-38 cells. *Biomed Pharmacother*, 2018; 103: 1678–87
21. Yoshimoto R, Mayeda A, Yoshida M et al: MALAT1 long non-coding RNA in cancer. *Biochim Biophys Acta*, 2016; 1859: 192–99
22. Yang Y, Zang S, Zhong C et al: Increased expression of the lncRNA PVT1 promotes tumorigenesis in non-small cell lung cancer. *Int J Clin Exp Pathol*, 2014; 7(10): 6929–35
23. Wu D, Chen X, Sun K et al: Role of the lncRNA ABHD11-AS1 in the tumorigenesis and progression of epithelial ovarian cancer through targeted regulation of RhoC. *Mol Cancer*, 2017; 16: 138
24. Wang H, Huo X, Yang X et al: STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4. *Mol Cancer*, 2017; 16: 136
25. Wu Q, Meng W, Jie Y et al: LncRNA MALAT1 induces colon cancer development by regulating miR-129-5p/HMGB1 axis. *J Cell Physiol*, 2018; 233: 6750–57
26. Ji Q, Liu X, Fu X et al: Resveratrol inhibits invasion and metastasis of colorectal cancer cells via MALAT1 mediated Wnt/ $\beta$ -catenin signal pathway. *PLoS One*, 2013; 8(11): e78700
27. Qiu G, Zhang XB, Zhang SQ et al: Dysregulation of MALAT1 and miR-619-5p as a prognostic indicator in advanced colorectal carcinoma. *Oncol Lett*, 2016; 12: 5036–42
28. Minhui Y, Shuangshuang W, Xiaoyan W et al: Construction of eukaryotic expression vector for functional gene fragment of noncoding RNA MALAT1 and its expression in human colon cancer SW620 cells. *J Trop Med*, 2010; 10: 248–57
29. Ayers D: Long non-coding RNAs: Novel emergent biomarkers for cancer diagnostics. *J Cancer Res*, 2013; 1: 31–35
30. Zhou M, Zhao H, Wang Z et al: Identification and validation of potential prognostic lncRNA biomarkers for predicting survival in patients with multiple myeloma. *J Exp Clin Cancer Res*, 2015; 34: 102
31. Chen Z, Yu C, Zhan L et al: LncRNA CRNDE promotes hepatic carcinoma cell proliferation, migration and invasion by suppressing miR-384. *Am J Cancer Res*, 2016; 6: 2299
32. Li H, Li Q, Guo T et al: LncRNA CRNDE triggers inflammation through the TLR3-NF- $\kappa$ B-cytokine signaling pathway. *Tumor Biol*, 2017; 39: 1010428317703821
33. Li Z, Tang Y, Xing W et al: LncRNA, CRNDE promotes osteosarcoma cell proliferation, invasion and migration by regulating Notch1 signaling and epithelial-mesenchymal transition. *Exp Mol Pathol*, 2018; 104: 19–25

## Conclusions

In this study, we verified the function of the regulatory network in LPS-induced MRC-5 cell, and we improved our understanding of the regulatory network of CRNDE in pneumonia, however, these results require validation in *in vivo* experiments. In future research we plan to verify the function of this regulatory network in mice and further explore downstream regulatory mechanisms of CRNDE, hoping to further elucidate the regulatory mechanisms and regulatory networks of CRNDE in pneumonia.

## Conflicts of interest

None.

34. Liu T, Zhang X, Yang Y et al: Increased expression of the long noncoding RNA CRNDE-h indicates a poor prognosis in colorectal cancer, and is positively correlated with IRX5 mRNA expression. *Onco Targets Ther*, 2016; 9: 1437–48
35. Chen L, Zhou Y, Li H: LncRNA, miRNA and lncRNA-miRNA interaction in viral infection. *Virus Res*, 2018; 257: 25–32
36. Ballantyne MD, McDonald RA, Baker AH: LncRNA/MicroRNA interactions in the vasculature. *Clin Pharmacol Ther*, 2016; 99: 494–501
37. Lei H, Gao Y, Xu X: LncRNA TUG1 influences papillary thyroid cancer cell proliferation, migration and EMT formation through targeting miR-145. *Acta Biochim Biophys Sin (Shanghai)*, 2017; 49(7): 588–97
38. Liu H, Deng H, Zhao Y et al: LncRNA XIST/miR-34a axis modulates the cell proliferation and tumor growth of thyroid cancer through MET-PI3K-AKT signaling. *J Exp Clin Cancer Res*, 2018; 37(1): 279
39. Debeb BG, Lacerda L, Anfossi S et al: miR-141-mediated regulation of brain metastasis from breast cancer. *J Natl Cancer Inst*, 2016; 108(8)
40. Tamagawa S, Beder LB, Hotomi M et al: Role of miR-200c/miR-141 in the regulation of epithelial-mesenchymal transition and migration in head and neck squamous cell carcinoma. *Int J Mol Med*, 2014; 33: 879–86
41. Quan B, Zhang H, Xue R: miR-141 alleviates LPS-induced inflammation injury in WI-38 fibroblasts by up-regulation of NOX2. *Life Sci*, 2019; 216: 271–78
42. Zheng Q, Peskoe SB, Ribas J et al: Investigation of miR-21, miR-141, and miR-221 expression levels in prostate adenocarcinoma for associated risk of recurrence after radical prostatectomy. *Prostate*, 2014; 74: 1655–62
43. Wang M, Hu M, Li Z et al: miR-141-3p functions as a tumor suppressor modulating activating transcription factor 5 in glioma. *Biochem Biophys Res Commun*, 2017; 490: 1260–67
44. Bhatia M, Sidhapuriwala JN, Ng SW et al: Pro-inflammatory effects of hydrogen sulphide on substance P in caerulein-induced acute pancreatitis. *J Cell Mol Med*, 2010; 12: 580–90
45. Thomas PS: Tumour necrosis factor-alpha: the role of this multifunctional cytokine in asthma. *Immunol Cell Biol*, 2001; 79: 132–40
46. Shi L, Wu L, Chen Z et al: MiR-141 activates Nrf2-dependent antioxidant pathway via down-regulating the expression of KEAP1 conferring the resistance of hepatocellular carcinoma cells to 5-fluorouracil. *Cell Physiol Biochem*, 2015; 35: 2333–48
47. Tatsumi N, Tokumitsu S, Nakano M et al: miR-141-3p commonly regulates human UGT1A isoforms via different mechanisms. *Drug Metabol Pharmacokin*, 2018; 33: 203–10
48. Van Jaarsveld MTM, Helleman J, Boersma AWM et al: miR-141 regulates KEAP1 and modulates cisplatin sensitivity in ovarian cancer cells. *Oncogene*, 2013; 32: 4284–93