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MicroRNA-146 protects A549 and H1975 cells from LPS-induced apoptosis and inflammation injury

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Pneumonia is an inflammatory condition affecting the lungs, in which pro-inflammatory cytokines are secreted. It has been shown that microRNA-146 (miR-146) is involved in the regulation of immune and inflammatory responses. The present study explored the protective effects of miR-146 overexpression on lipopolysaccharide (LPS)-mediated injury in A549 and H1975 cells. In this study, A549 and H1975 cells were transfected with miR-146 mimic or inhibitor, and then were subjected with LPS. Thereafter, cell viability, colony formation capacity, apoptosis, the release of proinflammatory factors, Sirt1 expression, and the expression of NF-κB and Notch pathway proteins were respectively assessed. As a result, miR-146 overexpression exerted protective functions on LPS-damaged A549 and H1975 cells, as evidenced by the increases in cell viability and colony number, the decrease in apoptotic cell rate, as well as the down-regulations of IL-1, IL-6, and TNF-α. Sirt1 can be positively regulated by miR-146. Furthermore, miR-146 overexpression blocked NF-κB and Notch pathways, while these blocking effects were abolished when Sirt1 was silenced. The findings in the current study indicated that miR-146 protected A549 and H1975 cells from LPS-induced apoptosis and inflammation injury. miR-146 exerted protective functions might be via up-regulation of Sirt1 and thereby blocking NF-κB and Notch pathways.

Keywords. Apoptosis; inflammatory cytokine; MicroRNA-146; NF-κB; Notch; pneumonia; Sirt1

1. Introduction

Pneumonia is inflammation of the lungs, most commonly caused by infection with viruses or bacteria. In the United States, pneumonia is the sixth most common cause of disease-related deaths. In addition, pneumonia is the most common hospital-acquired fatal infections. In developing countries, pneumonia along with diarrhea is the most common infectious cause of death in children (Abd-El-Fattah *et al.* 2013). During pneumonia, inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and granulocyte/macrophage colony-stimulating factor are released which lead to cell apoptosis, tissue necrosis, and microvascular dysfunction (Moldoveanu *et al.* 2009). Thus, suppression of inflammatory response and protection of cells from injury are the effective treatment strategies for pneumonia.

MicroRNAs (miRNAs) are small non-coding RNAs that are ∼22 nucleotides in length (Slezak-Prochazka *et al.* 2010). miRNAs act at the post-transcriptional level and modulate various cellular processes such as cell proliferation, invasion, and apoptosis (Bartel 2004). Role of miRNAs in the regulation of oncogenes and tumor suppressor genes has been studied in many types of cancer (Ferrando 2005; Garofalo *et al.* 2009; Shenouda and Alahari 2009; Kouhkan et al. 2011), including lung cancer (Shen *et al.* 2011), but their role in infectious lung disease such as pneumonia is not well studied. Few studies have indicated that miRNAs are closely related to the development and progression of pneumonia; for instance, miR-21, miR-155, and miR-197 have been shown to be overexpressed in pneumonia (Lu *et al.* 2009; O'Connell *et al.* 2007; Abd-El-Fattah *et al.* 2013).

miR-146 is widely expressed in mammalian cells (Schulte *et al.* 2013). Studies have indicated that miR-146 could directly combine with interleukin (IL)-1R-associated kinase (IRAK1) and TNF receptor-associated factor 6 (TRAF6), and thus participates in regulation of inflammatory responses (Xie 2013; Lee *et al.* 2016). Schulte et al showed that miR-146 targets the messengers of bacterial lipopolysaccharide

(LPS) to signal transduction components and thereby down-regulates cellular LPS sensitivity (Schulte *et al.* 2013). Based on these reports, in this study we explored whether miR-146 affected inflammatory response via regulating pro-inflammatory cytokines in pneumonia. In the present study, we determined effects of miR-146 on viability, apoptosis and inflammation in LPS-treated adenocarcinomic human alveolar basal epithelial cells (A549 cells) and in LPS-treated human lung adenocarcinoma cells (H1975 cells).

2. Materials and methods

2.1 *Cell culture and LPS treatment*

H1975 and A549 cell lines were provided by the Institute of Cell Biology (Shanghai, China). H1975 and A549 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U/mL and 100 mg/mL, respectively) and incubated at 37°C, 5% CO_2. The cells were treated with different concentrations (5, 10, and 15 μg/mL) of LPS under serum-free conditions for 12 h.

2.2 *Cell transfection*

miR-146 mimics, negative control (NC), and miR-146 inhibitor, as well as Sirt1 specific targeted siRNA (siRNA) were all purchased from GenePharma (Shanghai, China). Cell transfection was performed using HiPerFect transfection reagent according to the manufacturer's instructions (Qiagen, Germany). After 48 h of transfection, transfected cells were collected for use in the further investigations.

2.3 *MTT assay*

Cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2 5-diphenyl-2Htetrazolium bromide (MTT) colorimetric assay. In brief, the miR-transfected H1975 and A549 cells were seeded in 96-well plates with a density of 1×10^3 cells/well, and cells were then subjected with LPS for 12 h. After twice washing with phosphate buffer saline (PBS), 10 μL MTT solution (Sigma-Aldrich, St. Louis, MO, USA) with a final concentration of 5 mg/mL was added into each well. The plates were allowed for culturing at 37°C for 4 h, and then DMSO of 150 μL was added. The plates were placed in a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA), and the absorbance was measured after the plates were shaken for 10 min. Each experiment was performed three times.

2.4 *Clonogenic survival assay*

The miR-transfected cells were seeded in 6-well plates with a density of 1×10^3 cells/well, and were subjected with LPS for 12 h. After another 2 week incubation in normal conditions (without LPS stimulation), cells were fixed in 100% methanol and stained with 1% crystal violet (Sigma-Aldrich). Colonies containing more than 50 cells were defined as survivors.

2.5 *Apoptosis assay*

Cell apoptosis assay was performed to identify and quantify the apoptotic cells using Annexin V-FITC/PI apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China). The miR-transfected cells were seeded in 6-well plates with a density of 1×10^5 cells/well, and were subjected with LPS for 12 h. Treated cells were washed twice with cold PBS and resuspended in 200 μL binding buffer containing 10 μL Annexin V and 5 μL PI. The adherent and floating cells were combined and treated according to the manufacturer's instruction and measured with flow cytometer (Beckman Coulter, USA) to differentiate apoptotic cells (Annexin-V positive and PI-negative) from necrotic cells (Annexin-V and PI-positive).

2.6 *Quantitative reverse transcription polymerase chain reaction (qRT-PCR)*

Total RNA was isolated from transfected cells using Trizol reagent (Invitrogen) and treated with DNaseI (Promega). Reverse transcription was performed using Multiscribe RT kit (Applied Biosystems) and random hexamers or oligo (dT). The reverse transcription conditions were 10 min at 25°C, 30 min at 48°C, and a final step of 5 min at 95°C. For miR-146, the forward primer was 5′-GGGTGAGAACTGAATTCCA-3′ and the reverse primer was 5′-CAGTGCGTGTCGTGGAGT-3′. For Sirt1, the forward primer was 5′-TGCTGGCCTAATAGAGTGGCA-3′ and the reverse primer was 5′-CTCAGCGCCATGGAAAATGT-3′. For glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the forward primer was 5′-GCACCGTCAAGGCTGAGAAC-3′ and the reverse primer was 5′-TGGTGAAGACGCCAGTGGA-3′.

2.7 *Western blot analysis*

The proteins were extracted using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified

using BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. Primary antibodies were prepared in 5% blocking buffer at a dilution of 1:1000. Primary antibody was incubated with the membrane at 4 °C overnight, followed by wash and incubation with secondary antibody marked by horseradish peroxidase for 1 h at room temperature. After rinsing, polyvinylidene difluoride (PVDF) membrane-carried blots and antibodies were transferred into Bio-Rad ChemiDoc™ XRS system, and then 200 µL Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

2.8 *Statistical analysis*

All experiments were repeated three times. The results of multiple experiments are presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS 19.0 statistical software. The P-values were calculated using a one-way analysis of variance. A P-value of <0.05 was considered to be statistically significant.

3. Results

3.1 *LPS suppresses viability of A549 cells in dose-dependent manner*

LPS was used to mimic pneumonia-like situation in A549 cells. For this, different concentrations of LPS (5, 10, and 15 µg/mL) were used to suppress viability of A549 cells. As shown in figure 1, LPS suppressed A549 cells viability in dose-dependent manner, with statistically significant effects at 10 µg/mL ($P<0.05$) and at 15 µg/mL ($P<0.01$) concentrations. Thus, 10 µg/mL was selected as LPS-stimulating condition for use in the following investigations.

3.2 *Transfection efficiency of miR-146 in A549 and H1975 cells*

qRT-PCR was used to evaluate the transfection efficiency of miR-146 in A549 and H1975 cells transfected with miR-146 mimic, miR-146 inhibitor, or negative control (NC). As shown in figure 2A–B, miR-146 mimic significantly increased the expression of miR-146 ($P<0.01$ or $P<0.001$) and miR-146 inhibitor significantly decreased the expression of miR-146 (both $P<0.001$) in A549 and H1975 cells. These data indicated that the expression of miR-146 in A549 and H1975 cells were successfully overexpressed and suppressed by transfection.

3.3 *Expression of miR-146 and cell growth*

To test whether miR-146 can affect the response of A549 and H1975 cells to LPS, we measured the effects of miR-146 on viability of LPS-treated A549 and H1975 cells using MTT assay at 12, 24, 36, and 48 h (figure 3A–B). We found that overexpression of miR-146 enhanced cell viability compared with the negative control (NC) group. In contrast, miR-146 suppression decreased cell viability. These results indicate that overexpressing miR-146 efficiently increases viability of LPS-treated A549 and H1975 cells. Then, clonogenic survival assay was performed to crosscheck the impacts of miR-146 dysregulation on A549 and H1975 cells growth. As results shown in figure 3C–D, miR-146 overexpression increased the colony forming capacities of cell ($P<0.01$), and while miR-146 suppression could not significantly decrease the colony forming capacities ($P>0.05$).

Next, flow cytometry detection was performed to further determined whether the overexpression of miR-146 affect apoptosis in A549 and H1975 cells. Figure 3E–F shows that miR-146 overexpression significantly decreased cell apoptosis compared with the NC group ($P<0.01$), while miR-146 suppression significantly increased apoptosis ($P<0.05$ or $P<0.01$). Together with the result from MTT and clonogenic survival assays, we therefore briefly conclude that miR-146 overexpression might exert protective functions on LPS-induced impairment in A549 and H1975 cells growth.

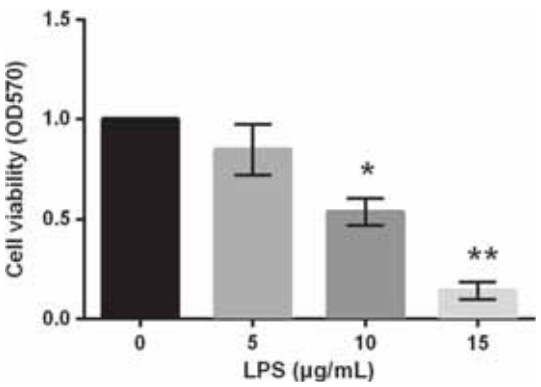

Figure 1. LPS inhibits viability of A549 cells. Viability of A549 cells in presence of different concentrations of LPS (5, 10, and 15 µg/mL). LPS: lipopolysaccharide. * $P<0.05$; ** $P<0.01$.

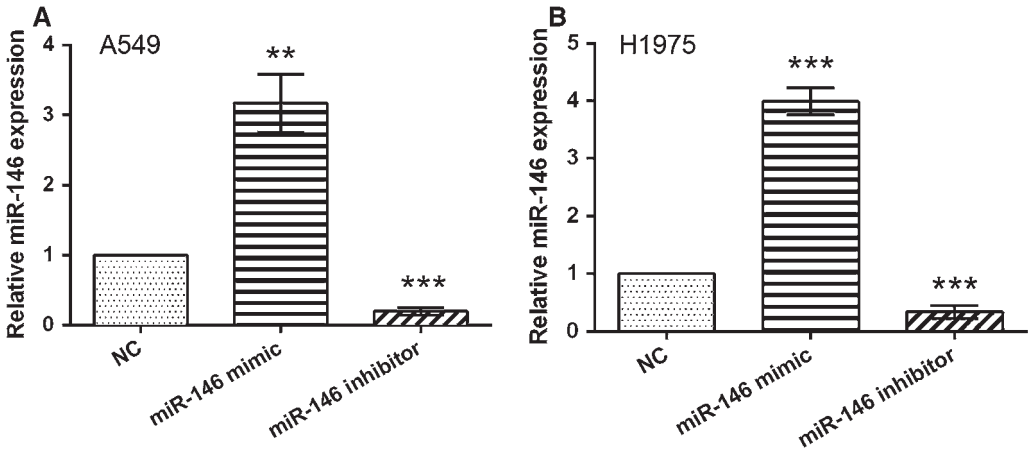

Figure 2. Transfection efficiency of miR-146 in A549 and H1975 cells. The transfection efficiency of miR-146 mimic, inhibitor, and negative control (NC) in (**A**) A549 and (**B**) H1975 cells was confirmed by qRT-PCR. GAPDH was used as an internal control. qRT-PCR: quantitative reverse transcription polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. ** $P<0.01$; *** $P<0.001$.

3.4 *Expression of miR-146 and the release of pro-inflammatory cytokines*

In pneumonia, cytokines are key regulators of inflammation. Therefore, we investigated the effect of miR-146 on the expression of pro-inflammatory cytokines, namely interleukin-1 (IL-1), IL-6, and TNF-α in LPS-treated A549 and H1975 cells. The expression levels of these cytokines in the transfected cells were detected using qRT-PCR and Western blot. As shown in figure 4A–D, overexpression of miR-146 decreased the expression of IL-1, IL-6, and TNF-α in A549 and H1975 cells, while knock-down of miR-146 significantly increased the expression of these cytokines. These data indicate that miR-146 overexpression can protect A549 and H1975 cells from LPS-induced inflammation response by negatively regulating pro-inflammatory cytokine secretion.

3.5 *Regulatory effects of miR-146 on Sirt1 expression levels*

We then explored the mechanism underlying the protective functions of miR-146 on apoptosis and inflammation in A549 and H1975 cells. We measured the expression of Sirt1, as it has been widely identified as an anti-apoptotic and anti-inflammatory molecule (Hattori and Ihara 2016; Hwang *et al.* 2013). qRT-PCR and western blot analytical results showed that overexpression of miR-146 significantly up-regulated Sirt1 ($P<0.05$ or $P<0.001$), while suppression of miR-146 significantly down-regulated Sirt1 ($P<0.05$ or $P<0.01$; figure 5A–D). These findings altogether imply that miR-146 overexpression inhibits LPS-induced apoptosis and inflammation injury via up-regulating Sirt1.

3.6 *Expression of miR-146 and NF-κB and Notch signalling pathways*

Finally, we studied the effects of overexpression of miR-146 on signalling pathway proteins involved in apoptosis and inflammatory responses. qRT-PCR and Western blot analysis were used to measure the expression of phosphorylated/total IκBα (p/t-IκBα), phosphorylated/total p-65 (p/t-p65), Notch 1, Notch 2, and Jagged 1 (JAG 1) in A549 cells. The results showed that overexpression of miR-146 markedly decreased the expression levels of p/t-IκBα, p/t-p65, Notch 1, and JAG 1 in LPS-treated A549 cells as compared to the negative control (NC) ($P<0.05$ or $P<0.001$; figure 6A and B). miR-146 overexpression did not significantly decreased Nocth 2 levels. In contrast, suppression of miR-146 slightly increased the expression levels of p/t-IκBα, Notch 2, and JAG 1 as compared to the NC group (figure 6A and B), although the increases of Notch 2, and JAG 1 levels did not reach statistical significance.

Furthermore, we confirmed the effect of miR-146 on Sirt1 by silencing the expression of Sirt1 using Sirt1 specific targeted siRNA. The results showed that miR-146 overexpression did not decrease the expression levels of p/t-IκBα, p/t-p65, Notch 1, and JAG 1 under LPS-treated conditions when Sirt1 was silenced (figure 6A and B). These data indicate that miR-146 blocked nuclear factor-κB (NF-κB) and Notch signalling pathways in A549 cells in Sirt1-dependent manner.

4. Discussion

This study explored the protective effects of miR-146 on A549 and H1975 cells against LPS mediated damage. At first we showed that overexpression of miR-146 increases

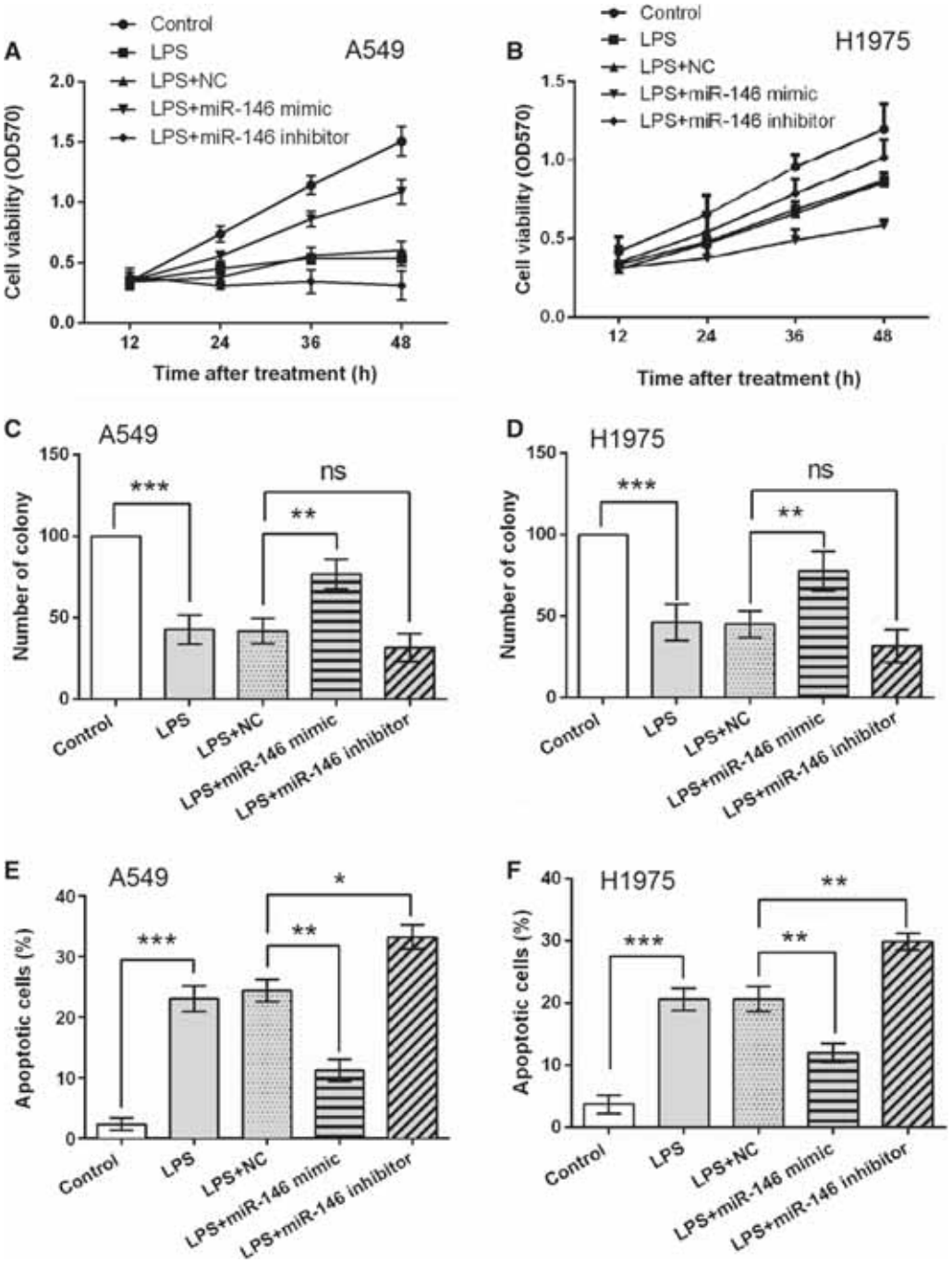

Figure 3. Expression of miR-146 and cell growth of A549 and H1975 cells. A549 and H1975 cells were transfected with miR-146 mimic, miR-146 inhibitor, or negative control (NC), and then were subjected with 10 μg/mL LPS for 12 h. (**A**–**B**) Viability of A549 and H1975 cells was measured at 12, 24, 36, and 48 h after LPS exposure using MTT assay. (**C**–**D**) Colony formation capacity of A549 and H1975 cells were analysed by clonogenic survival assay. Data presented as actual number of colonies ± SD in control wells. (**E**–**F**) Apoptotic cell rate was measured by using flow cytometry. LPS: lipopolysaccharide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2 5-diphenyl-2Htetrazolium bromide. ns, no significance; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

viability and colony formation capacities of LPS-treated cells (figure 3A–D). Then we analysed cell apoptosis and found that overexpression of miR-146 inhibited LPS-induced apoptosis (figure 3E–F). Furthermore, we analysed the expressions of pro-inflammatory cytokines (IL-1, IL-6, and TNF-α), and found that miR-146

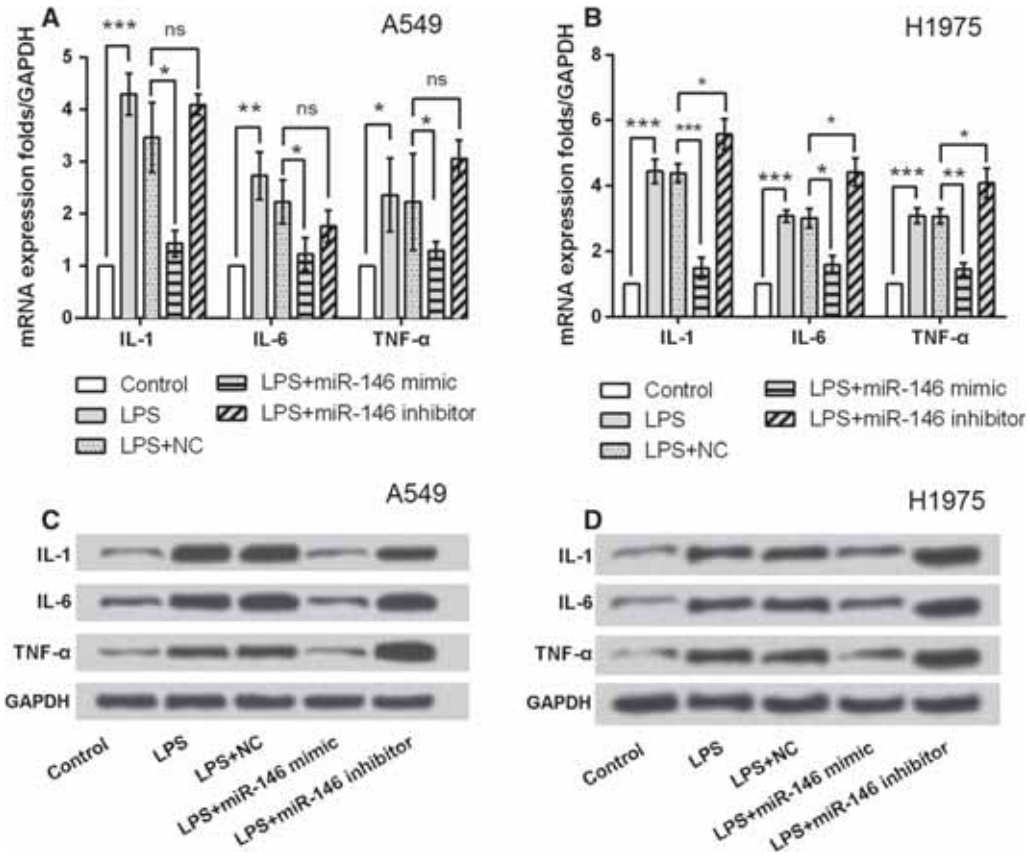

Figure 4. Expression of miR-146 and the release of pro-inflammatory cytokines in A549 and H1975 cells. A549 and H1975 cells were transfected with miR-146 mimic, miR-146 inhibitor, or negative control (NC) and were subjected with 10 μg/mL LPS for 12 h. The expression levels of IL-1, IL-6, and TNF-α in the LPS-treated A549 and H1975 cells were detected using (**A**) qRT-PCR and (**B**) Western blot analysis. GAPDH served as internal control. IL: interleukin; qRT-PCR: quantitative reverse transcription polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LPS: lipopolysaccharide; TNF: tumor necrosis factor. ns, no significance; * P<0.05; ** P<0.01; *** P<0.001.

overexpression decreased the expression of these cytokines (figure 4). The mechanism underlying this anti-apoptotic and anti-inflammatory effects was found to be up-regulation of Sirt1 by miR-146 (figure 5). Finally, we studied signalling pathway that could be playing potential role behind these effects. The results showed that overexpression of miR-146 markedly decreased expression levels of NF-κB and Notch signalling pathways, which explains the role of miR-146 in inflammatory response (figure 6).

miR-146 family consists of miR-146a and miR-146b. It has been reported that aberrant expression of miR-146a is involved in the development and progression of many types of cancers, including lung cancer (Kamali *et al.* 2016; Li *et al.* 2016; Meshkat *et al.* 2016). In non-small cell lung cancer (NSCLC), miR-146a has been shown to inhibit cell growth and cell migration (Chen *et al.* 2013). Wang and Liu reported that miR-146a significantly decreased viability of A549 cells in lung cancer patients (Wang and Liu 1995). In our study, we used the same cell line (A549) and another lung adenocarcinoma cell line (H1975) demonstrated that overexpression of miR-146 increases viability and colony formation capacity of LPS-treated A549 and H1975 cells. This contradiction may be caused by the difference of stimulus, of which cell subjected with. Further investigations are required to confirm this hypothesis.

In pneumonia, inflammatory cytokines are released, resulting into induction of apoptosis (Moldoveanu *et al.* 2009). Therefore, we investigated whether the increased expression of miR-146 could inhibit apoptosis in lung cells. Confirming our theory, the flow cytometry results showed that overexpressing miR-146 inhibits apoptosis in A549 and H1975 cells treated with LPS. Similar findings have been reported in previous studies. Wang and Liu demonstrated that increased expression of miR-146a significantly decreased apoptosis in A549 cells (Wang and Liu 1995). Chen et al reported that miR-146a inhibits apoptosis in five NSCLC cell lines including H358, H1650, H1975, HCC827, and H292 (Chen *et al.* 2013). Furthermore, we also studied the mechanism behind this anti-apoptotic effect. Sirt1 is a nicotine adenine dinucleotide (NAD)-dependent deacetylase

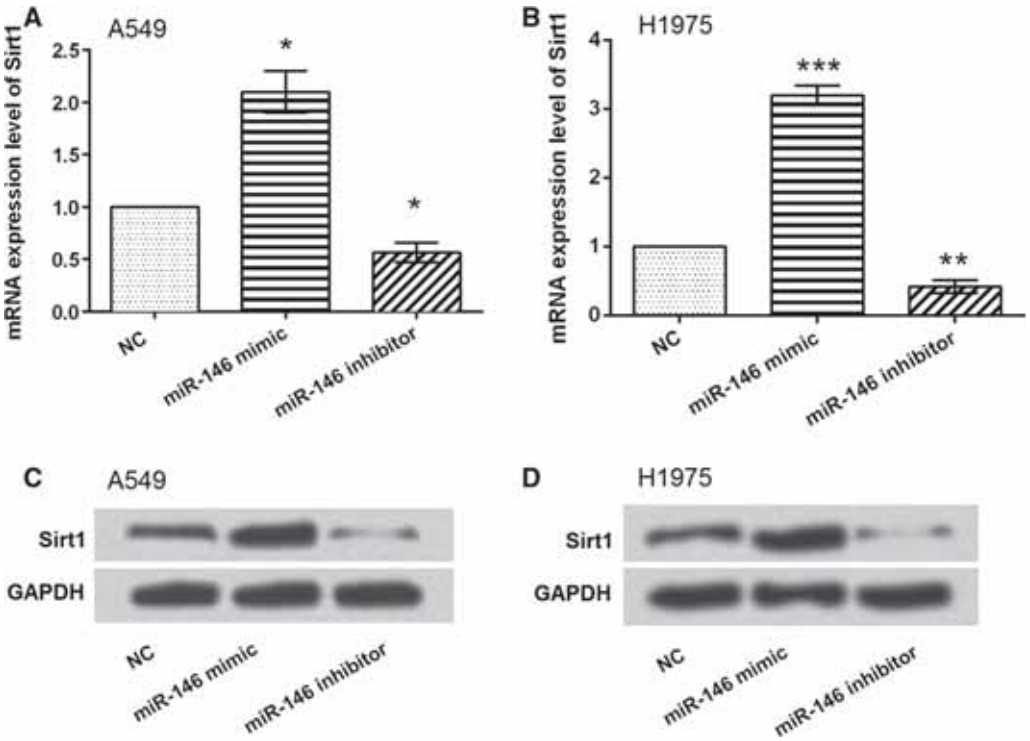

Figure 5. Regulatory effects of miR-146 on Sirt1 expression levels. A549 and H1975 cells were transfected with miR-146 mimic, miR-146 inhibitor, or negative control (NC). (**A**–**B**) qRT-PCR and (**C**–**D**) Western blot analysis were used to measure the effect of miR-146 on Sirt1 expression in A549 and H1975 cells. GAPDH served as internal control. qRT-PCR: quantitative reverse transcription polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LPS: lipopolysaccharide. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

which regulates apoptosis in response to oxidative and genotoxic stress (Haigis and Guarente 2006; Longo and Kennedy 2006). It has been widely identified as an anti-apoptotic and anti-inflammatory molecule (Hattori and Ihara 2016; Hwang *et al.* 2013). We measured the expression level of Sirt1 and found that overexpression of miR-146 significantly up-regulated Sirt1 in A549 and H1975 cells. Together these data indicate that increased expression of miR-146 in A549 and H1975 cells inhibits apoptosis and inflammation via up-regulating Sirt1. Other studies have also explored effect of miR-146 on Sirt1 expression. In two different studies, miR-146 was shown to directly target Sirt1 in pancreatic cells, which resulted in decreased apoptosis (Ramachandran *et al.* 2011; Lovis *et al.* 2008).

Cytokines are key regulators of inflammation in pneumonia. It has been showed that miR-146 is involved in inflammation in other tissues (Nakasa *et al.* 2008; Jin *et al.* 2009). Therefore, we assessed the impact of miR-146 on the expression of pro-inflammatory cytokines, namely IL-1, IL-6, and TNF-α. It has been established that IL-1 is involved in inflammation, pain and autoimmune conditions (Ren and Torres 2009). IL-6 is involved in inflammation and infection responses, as well as regulation of metabolic, regenerative, and neural processes (Scheller *et al.* 2011). TNF-α is found in increased concentrations in acute and chronic inflammatory conditions, such as infection, rheumatoid arthritis, trauma, and sepsis (Popa *et al.* 2007). In our study, overexpression of miR-146 decreased the expression of these cytokines in A549 and H1975 cells. Similar results have been reported in previous studies. Gu *et al.* demonstrated that miR-146a suppresses IL-1-dependent inflammatory responses in the intervertebral disc (Gu *et al.* 2015). Another study showed that increased expression of miR-146a decreased the expression of IL-6 in LPS-stimulated RAW264.7 macrophage cells (He *et al.* 2014). Eun-Ah and Jena showed that overexpression of miR-146a reduced the levels of TNF-α in primary human retinal microvascular endothelial cells (Eun-Ah Y and Jena JS 2016). Zeng *et al.* also demonstrated that miR-146a mimic significantly suppressed LPS-mediated TNF-α, IL-6, and IL-1β induction in NR8383 cells (Zeng *et al.* 2013). All these findings suggest that miR-146 negatively regulates pro-inflammatory cytokines and thereby inhibit inflammatory response in pneumonia cells.

Finally, we explored the molecular mechanisms involved in the regulation of these pro-inflammatory cytokines. We evaluated the expression levels of p/t-IκBα, p/t-p65, Notch 1, Notch 2, and JAG 1. NF-κB is a transcription factor, playing important roles in cell proliferation, apoptosis, immunity, and inflammation. Phosphorylation of IκBα and

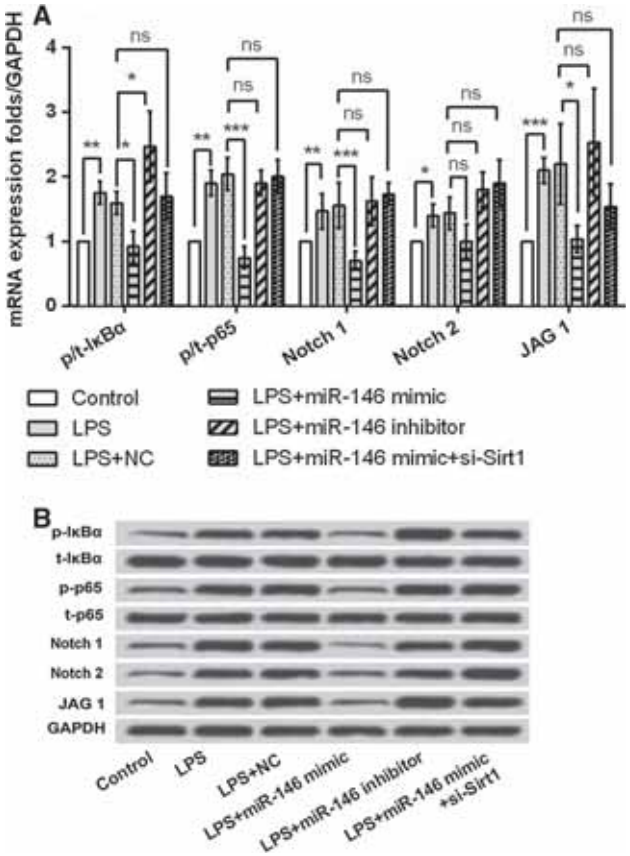

Figure 6. Expression of miR-146 and NF-κB and Notch signalling pathways. A549 cells were transfected with miR-146 mimic, miR-146 inhibitor, their negative control (NC), or Sirt1 siRNA (si-Sirt1) and then were subjected with 10 μg/mL LPS for 12 h. (**A**) qRT-PCR and (**B**) Western blot analysis were used to measure the expression levels of p/t-IκBα, p/t-p65, Notch 1, Notch 2, and JAG 1 in A549 cells. GAPDH served as internal control. p/t-IκBα: phosphorylated IκBα; p/t-p65: phosphorylated p-65; JAG 1: Jagged 1; qRT-PCR: quantitative reverse transcription polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LPS: lipopolysaccharide. ns, no significance; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

p-65 activates NF-κB (Viatour *et al.* 2005). As deregulation of NF-κB and IκBα phosphorylations are main characteristics of chronic inflammatory diseases, novel drugs are targeting these signalling pathways. There are four Notch receptors (notch 1-4) and five Notch ligands (JAG 1, JAG 2, delta-like1, delta-like3, and delta-like 4) (Quillard and Charreau 2013). Few reports have indicated that Notch signalling plays crucial role in inflammation (Okamoto *et al.* 2008; Niranjan *et al.* 2008). In the present study, we demonstrated that overexpression of miR-146 decreases the expression of p/t-IκBα, p/t-p65, Notch 1, and JAG 1 in A549 cells. Furthermore, we demonstrated that silencing the Sirt1 expression reversed the inhibitory effect of miR-146 overexpression on the expression levels of p/t-IκBα, p/t-p65, Notch 1, and JAG 1. These finding suggests that miR-146 alleviates inflammation by up-regulating Sirt1 and thus blocking NF-κB and Notch signalling pathways.

In conclusion, this study revealed that miR-146 protected A549 and H1975 cells from LPS-induced apoptosis and inflammation injury. miR-146 exerted protective functions might be via up-regulation of Sirt1 and thereby blocking NF-κB and Notch signalling pathways. The findings in the present study suggest the potential role of miR-146 as a novel drug target for pneumonia.

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