ORIGINAL ARTICLE

The critical function of miR-1323/Il6 axis in children with Mycoplasma pneumoniae pneumonia

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Abstract

Objective: Mycoplasma pneumoniae pneumonia (MPP) is a common respiratory infection in children. Tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), and IL-6 have correlation with Mycoplasma pneumoniae lung infection and MPP pathogenesis.
Method: miRNAs participate in the pathogenesis of various diseases by regulating the development and differentiation of the immune cell. Blood was collected and total RNA was isolated. miRNA microarrays were performed to identify differentially expressed miRNAs in MPP patients. The levels of relative miRNAs and mRNAs were evaluated by qRT-PCR.
Results: There are 23 differentially expressed miRNAs in MPP children’s plasma, 15 miRNAs had enhanced expression and 8 had depressed expression. MPP patients showed lower mir-1323 level in blood samples than healthy controls. MPP patients with pleural effusion had much higher Il6 and Il17a mRNA levels than those without pleural effusion. The expression level of Il6 had a negative correlation with miR-1323 level. In the human THP-1 cell line, the level of miR-1323 was significantly reduced through lipopolysaccharides treatment. In THP-1 cells, overexpression or silencing of miR-1323 significantly reduced or promoted Il6 expression.
Conclusion: In conclusion, miR-1323 targets the mRNA of Il6 and inhibits the expression of Il6. The pathogenesis of MPP inhibits the expression of miR-1323 in macrophages, triggers the overexpression of Il6, and enhances inflammation response.

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Introduction

The smallest free-living prokaryote is mycoplasma. In a pool of 16 different kinds of human mycoplasmas, 6 of them can cause disease, and the predominant pathogen is Mycoplasma pneumoniae. Mycoplasma pneumoniae caused Mycoplasma pneumoniae pneumonia (MPP) is a common respiratory infection in children. According to statistical data, mycoplasma pneumonia accounts for 10%–40% of community-acquired pneumonia (CAP) in children. The infection rate of Mycoplasma pneumoniae in the youth has elevated, and patients with severe mycoplasma pneumonia and refractory mycoplasma pneumonia have increased. It is currently known that Mycoplasma pneumoniae can continuously damage the respiratory epithelium and cilia through the induction of immune response, and the host cannot effectively clear the pathogen. Severe MPP cases will give rise to numerous complications and eventually develop into a severe life-threatening pneumonia.

The human body produces a variety of inflammatory factors by activating immune cells after a Mycoplasma pneumoniae infection. The immune response mediates and regulates immune function and inflammatory response. The inflammatory factors accumulate locally and are gradually spread from upper respiratory tract to the lower respiratory tract. The clinical manifestations are bronchiolitis, pneumonia, etc.; in severe cases, the brain, heart, liver, and kidneys of the child may be involved, causing complications such as encephalitis, myocarditis, and hepatitis. Research showed that tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17) and IL-6 had correlation with Mycoplasma pneumoniae lung infections and MPP pathogenesis. However, the specific molecular mechanism of MPP stimulating inflammation is unknown.

In recent years, microRNA (miRNA) has been recognized by researchers as a new class of regulatory gene molecules. miRNA is a kind of evolutionarily conservative endogenous non-coding short chain small RNA. It has been shown that it can participate in the occurrence and development of various diseases. There are few reports of miRNA expression in MPP. miR-1323 is widely reported to participate in the pathogenesis of several different cancers, such as breast cancer, squamous cell carcinoma, and lung cancer. Based on our microarray analysis result, the expression of miR-1323 was depressed in children with MPP. Thus, this research aimed to investigate miRNA-1323 expression and possible function in children with MPP.

Methods

Patient characteristics

From March 2019 to April 2020, 26 children with MPP were recruited in this research. Exclusion criteria include current immunomodulator and immunosuppressive agent usage, recurrent pneumonia, premature delivery, and immunodeficiency. 9 children in these participants had pleural effusion, and 31 healthy children were recruited as control in the same period. Inclusion criteria of healthy control include no respiratory tract infection, no chronic infectious disease, no immune system disease history, no allergies, and no other immunity-related disease. The healthy controls have also been tested to exclude those with potential MPP infection by PCR on nasal swabs. This research was approved by the ethics committee of Cangzhou Central Hospital and corresponding informed consents were signed by all the participants’ parents or guardians.

Mycoplasma pneumoniae infection was diagnosed by serologic testing and PCR from nasopharyngeal secretion. The clinical and demographic data of participants were collected through questionnaires. Peripheral blood samples were collected for laboratory examination.

THP-1 cell line

The human THP-1 cell line, derived from the peripheral blood of a 1-year-old male with acute monocytic leukaemia, was purchased from ATCC. THP-1 cells were cultured in RPMI 1640 medium (Thermo Fisher, USA), supplemented with 10% fetal bovine serum (Invitrogen, USA), 100 U/mL penicillin-streptomycin (Sigma-Aldrich, USA), and 2 mM L-glutamine with 5% CO₂ at 37 °C. 0.1 μg/mL LPS (Sigma-Aldrich) was employed for stimulating THP-1 cells during the relative period.

Microarray analysis of miRNAs in plasma

Plasma sample (200 μL) was mixed with Trizol (Ambion, USA) (750 μL) to isolate total RNA. Microarray hybridization, data generation and normalization were performed by the Kangchen Biological Engineering Co. Ltd. Human miRNA chip (miRCURY™, Exiqon, Denmark) with 3100 miRNA probes employed. Quantile algorithm was used for normalization. When a false discovery rate of ≤0.05 and p-value <0.05, miRNA was thought to be differentially expressed. If the expression of a miRNA had more than a two-fold difference between MPP children and healthy control, this miRNA was also thought to be differentially expressed.

qRT-PCR

Total RNA of the PBMCs from the children with MPP was isolated by Trizol (Invitrogen, USA). Reverse transcription PCR was performed by the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, USA). qRT-PCR was performed by the TaqMan Universal PCR Master Mix kit (Applied Biosystems). Actb was used as an endogenous control. Primers used in this experiment were the following:

| Gene | Primer Sequence |
|------|-----------------|
| human IL6 | 5'-AGACAGCCACTACCTTCTTCCAG-3' |
| human IL6 R | 5'-TTCTGCCAGTGGCCTTGGCCTG-3' |
| human IL17 | 5'-CCGGACTGTGTGATGTCA-3' |
| human IL17 R | 5'-CTCTGCGTGTGACAGATT-3' |
| human TNF | 5'-CTCTTCCTGCTGCTGACATT-3' |
| human TNF R | 5'-ATGGGCTCAAGGGTGGTAC-3' |
| human IL1B | 5'-CCACAGACCTTCTCCAGG-3' |
| human IL1B R | 5'-GTGAGTTCAGTGATCTCAGG-3' |
| human MI-R-1323 | 5'-AAACCTGGGAGATT-3' |
| human MI-R-1323 R | 5'-GAAGATCTGCTGATATC-3' |
| human MI-R-98-5P | 5'-GAGGTAGTAAGTTGTATT-3' |
| human MI-R-98-5P R | 5'-GAGGTAGTAAGTTGTATT-3' |
15 miRNAs had enhanced expression and 8 had depressed expression. Based on the result of microarray analysis, the most down-regulated miRNAs were miR-1323, miR-98-5p, and miR-152-3p.

**miR-1323 expression is significantly depressed in children with MPP**

To further confirm the result of microarray analysis, the levels of miR-1323, miR-98-5p, and miR-152-3p were also evaluated through qRT-PCR. As shown in Fig. 2A, when compared with healthy controls, patients with MPP had dramatically lower levels of miR-1323, miR-98-5p, and miR-152-3p in blood samples. Among 26 children with MPP, 9 of them had pleural effusion. Meanwhile, compared with MPP cases lacking pleural effusion, the patients who did have pleural effusion presented lower mir-1323 and mir-98-5p levels; but mir-152-3p has no significant difference (Fig. 2B).

**Il6 is the targeted gene regulated by miRNA-1323**

According to the Target Scan 7.1 database, Il6 mRNA is one of the direct targets of miR-1323 in the PBMCs (Fig. 3A). As shown in Fig. 3B, an activated inflammation response caused by MPP elevated Il17a and Il6 mRNA levels in MPP patients. Pleural effusion is an indicator and clinical manifestation of severe inflammation. Compared with children without pleural effusion, children with MPP and pleural effusion had much higher mRNA levels of Il6 and Il17a in blood samples (Fig. 3C). Through the Pearson Correlation test, the expression level of Il6 was confirmed to have a significant negative correlation with the level of miR-1323 (Fig. 3D).

**miR-1323 specially regulates Il6 expression in THP-1 cells**

Macrophages play a crucial role in the excessive inflammation caused by pneumonia infection. In this study, LPS stimulation in vitro was used to mimic M. pneumoniae infection, as it is well recognized that membrane lipopolysaccharides are immuno-stimulants exerting as lipopolysaccharides (LPS) and play a crucial role in the pathogenesis of inflammatory responses upon M. pneumoniae infection. In THP-1 cells, miR-1323 expression was inhibited by the LPS treatment, with the effect of LPS enhanced by increased time of treatment (Fig. 4A). As shown in Fig. 4B, the expression of Il6, Tnf, and Il1b were all enhanced by LPS, but only Il6 expression was elevated by the inhibition of miR-1323 expression (Fig. 4B). A decreased level of miR-1323 was also shown in Fig. 4B. Meanwhile, an enhanced expression of miR-1323 declined the mRNA level of Il6 after LPS treatment but had no influence on Tnf and Il1b expression (Fig. 4C). An elevated level of miR-1323 was also shown in Fig. 4C.

**Discussion**

It is currently believed that MPP is a combination of a direct pathogen invasion and immune injury. Studies demonstrate that the adhesion and proliferation of *Mycoplasma pneumoniae* to the host respiratory mucosal epithelial cells is the

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**Figure 1**  Total miRNAs profiling from microarray analysis. Heat map and cluster analysis of miRNA expression. Individual patient samples are shown in columns and miRNAs. Individual patient samples are shown in columns and miRNAs in rows. Of all differentially expressed miRNAs, 15 miRNAs were up-regulated and 8 miRNAs down-regulated.

MPP, mycoplasma pneumoniae pneumonia.

human mir-152-3p R: GAAACATGTCCTGCTATCTCT
human Actb F: AGCTTGCATCCAGGCTGCTAT
human Actb R: TTAATGTACGAGCAGATTTCCGC

**Statistical analysis**

SPSS Statistics Version 22.0 software was employed to perform statistical analysis. Values were expressed as n (percentage, %) or mean ± SD. Data were statistically analyzed using two-sided Student’s t-test and the Pearson Correlation test. The chi-square test was used for analyzing non-parametric data. p value less than 0.05 was considered to be statistically significant.

**Results**

**Participants characteristics**

Clinical and demographic data in both MPP group and control group were collected and showed in the supplementary Table S1. When compared with children in the control group, those with MPP presented longer duration of fever, enhanced neutrophils number, and elevated lactate dehydrogenase and C-reactive protein levels. The lymphocytes subgroups were also examined and the CD19+ CD23+ cell proportion was dramatically elevated in children with MPP. The concentration of *Mycoplasma pneumoniae* specific IgG was also increased.

**Altered miRNA profiles in the blood of MPP patients**

Through microarray analysis, miRNA expression in MPP patients’ blood samples were profiled and the heat map and cluster analysis are shown in Fig. 1. A total of 23 miRNAs had significantly altered expression in MPP patients,
Figure 2  Comparisons of multiple miRNA between MPP cases and healthy controls.  
a, Levels of miRNA in in the PBMCs from the MPP patients and healthy controls; b, Levels of miRNA in MPP patients with pleura effusion and MPP patients without pleura effusion. Error bars indicate standard error. All qRT-PCR data are presented as the fold induction relative to the Actb mRNA level.  
MMP, mycoplasma pneumoniae pneumonia.

Figure 3  Il6 is the targeted gene regulated by miRNA-1323. 
a, The binding site of miR-1323 in the Il6 mRNA; b, Expression of Il6 and Il17a in the PBMCs from the children with MPP; c, Expression of Il6 and Il17a in MPP cases with and without pleural effusion; d, Correlation between the concentration of Il6 and miR-1323. All qRT-PCR data are presented as the fold induction relative to the Actb mRNA level.  
MMP, mycoplasma pneumoniae pneumonia.
Mycoplasma pneumoniae can firmly adhere and invade the epithelial cells to escape the body's immune mechanism or drug treatment. It can persist in the respiratory tract for several months, making the patient a chronically infected person or an asymptomatic carrier.16

MPP has become a common disease in children. Due to the long course of disease and severe symptoms, it can easily cause a variety of extrapulmonary complications.17 During the pathogenesis of MPP, inflammatory mechanisms also play an important role.18 Immune cells and cytokines dominate in immune injury, and the molecular mechanism that causes pathological changes is not very clear and is the focus of current research.

Recently, studies have shown that the miRNA participates in the occurrence and development of diseases by regulating immune cell development and differentiation. In acute lung injury, the expression of multiple miRNAs in the lungs is dynamically regulated, miR-16 is up-regulated and miR-150 is down-regulated.18,19 In mice, overexpression of miR-181a promotes B lymphocyte differentiation and reduces circulating T lymphocytes.20 The transformation of T cells is promoted by miR-181a, miR-146a and miR-146b, leading to a pro-inflammatory response.21 miR-155 participates in regulating T helper cell differentiation and mediating T cells to participate in the immune response.22 In monocytes, miR-155 responds to viruses and bacteria infection and reduces the inflammatory response.23 The let-7 miRNAs inhibit the expression of IL-13 and regulate IL-13 secretion.24 In inflammation caused lung damage, the miR-127 expression is down-regulated, and an enhanced miR-127 expression can inhibit the release of cytokines by macrophages.25

In this study, microarray analysis found that there were differences in the expression of miRNA in the plasma of children with MPP, suggesting that miRNA may participate in the occurrence and development of MPP. There were 23 differentially expressed miRNAs in MPP children's plasma, 15 miRNAs had enhanced expression and 8 had depressed expression. The most down-regulated miRNAs were miR-1323, miR-98-5p, and miR-152-3p.

It is thought that a strong inflammatory reaction in children with severe MPP can trigger a systemic inflammatory reaction and pleural effusion.26 According to relevant reports, the infection rate of severe MPP with pleural effusion is as high as 50%.27 For children with severe MPP and pleural effusion, cephalosporin or penicillin are often used in clinical anti-infective treatment.28 In this research, among 26 children with MPP, 9 of them had pleural effusion. Levels of mir-1323 and mir-98-5p were significantly lower in MPP patients with pleural effusion than those with no pleural effusion.

Research showed that IL-17, IL-6 and TNF-α are indicators commonly used in clinics to reflect inflammation.29 It is widely used in infectious diseases. Altered IL17, IL6, and TNF-α expressions are important for the occurrence of Mycoplasma pneumoniae lung infection and MPP pathogenesis.30 Serum IL-17 is an inflammatory mediator that has a certain effect on the immune response of cells induced by Mycoplasma pneumoniae. IL-17 has played a defensive role in the lung infection of Mycoplasma pneumoniae, which can improve the body’s ability to eliminate pathogens and promote neutrophils to aggregate.30 Serum IL-6 is a highly active immuno-regulatory factor that participates in pathological processes of lung inflammation. By detecting the expression of IL17 and IL6 levels in a patient's serum, the patient’s condition can be accurately assessed.

Compared with controls, children with MPP had lower mRNA levels of IL6 and IL17a. Compared with children without pleural effusion, children with MPP and pleural effusion had much higher mRNA levels of IL6 and IL17a. According to the Target Scan 7.1 database, IL6 is one of the direct targets of miR-1323. The expression level of IL6 also showed a significant negative correlation with the level of miR-1323.

After Mycoplasma pneumoniae invades the lower respiratory tract, it stimulates epithelial cells and macrophages to release a variety of cytokines such as IL-1β, IL-4, IL-6, IL-8, IL-18, IFN-γ, activates specific and non-specific...
immune cells, and causes excessive immune inflammation in the pathogenesis of MPP. In severe cases, activated macrophages can cause damage to multiple tissues and organs of the body, causing hemophagocytic syndrome. Kurai et al. found that the levels of IL-17, IL-6, TNF-α and IL-4 in the alveolar lavage fluid of Mycoplasma pneumoniae infected mice increased, aggravating the lung inflammation caused by neutrophils.

Based on these results, we used LPS to stimulate THP-1 cells and explored the expression of miR-1323, Il6 and various cytokines in LPS stimulated THP-1 cells. The expression of miR-1323 in THP-1 cells was inhibited by LPS treatment. After the administration of LPS, the expression of Il6, Tnf, and Il1b were all enhanced in THP-1 cells. However, only the expression of Il6 was inhibited by miR-1323 overexpression and promoted through the inhibition of miR-1323 expression.

Cytokines play an important role in the occurrence and development of MPP, and its in-depth study is conducive in exploring the specific pathogenic mechanism of MPP. In recent years, with the increase in the incidence of MPP and with more and more severe and refractory cases, the study of its pathogenesis is conducive to an early clinical identification and effective treatment.

Conclusion
In conclusion, miR-1323 targets the mRNA of Il6 and inhibits the expression of Il6. The pathogenesis of MPP inhibits the expression of miR-1323 in macrophages, triggers the overexpression if Il6 and enhances inflammation response. It should be noted that blood samples in the current study were not collected at any particular phase or stage of MPP, and it would be important to investigate the temporal expression profile of these miRNAs at different phases of the disease in the future. Also, the sample size is relatively small in this study, and more samples could be analyzed to verify the findings.

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Conflict of interest
The authors declare no conflicts of interest.

Appendix A. Supplementary data
Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jped.2020.11.004.

References
1. Waites KB, Crabb DM, Bing X, Duffy LB. In vitro susceptibilities to and bactericidal activities of garenoxacin (BMS-284756) and other antimicrobial agents against human mycoplasmas and ureaplasmas. Antimicrob Agents Chemother. 2003;47:161–5.
2. Youn YS, Lee KY, Hwang JY, Rhim JW, Kang JH, Lee JS, et al. Difference of clinical features in childhood Mycoplasma pneumoniae pneumonia. BMC Pediatr. 2010;10:48.
3. Yan Y, Wei Y, Jiang W, Hao C. The clinical characteristics of corticosteroid-resistant refractory Mycoplasma Pneumoniae pneumonia in children. Sci Rep. 2016;6:39929.
4. Chen Y, Tian WM, Chen Q, Zhao HY, Huang P, Lin ZQ, et al. Clinical features and treatment of macrolide-resistant Mycoplasma pneumoniae pneumonia in children. Zhongguo Dang Dai Er Ke Za Zhi. 2018;20:629–34.
5. Radisic M, Torn A, Gutierrez P, Defranchi HA, Pardo P. Severe acute lung injury caused by Mycoplasma pneumoniae: potential role for steroid pulses in treatment. Clin Infect Dis. 2000;31:1507–11.
6. Shimizu T. Inflammation-inducing Factors of Mycoplasma pneumoniae. Front Microbiol. 2016;7:414.
7. Chaudhry R, Ghosh A, Chandola A. Pathogenesis of Mycoplasma pneumoniae: an update. Indian J Med Microbiol. 2016;34:7–16.
8. Kawai Y, Miyashita N, Kato T, Okimoto N, Narita M. Extra-pulmonary manifestations associated with Mycoplasma pneumoniae pneumonia in adults. Eur J Intern Med. 2016;29:e9–10.
9. Fan H, Lu B, Yang D, Zhang D, Shi T, Lu G. Distribution and expression of IL-17 and related cytokines in children with Mycoplasma pneumoniae Pneumonia. Jpn J Infect Dis. 2019;72:387–93.
10. Zhang F, Yang C, Xing Z, Liu P, Zhang B, Ma X, et al. LncRNA GAS5-mediated miR-1323 promotes tumor progression by targeting TP53INP1 in hepatocellular carcinoma. Onco Targets Ther. 2019;12:4013–23.
11. Xu Y, Liu M. MicroRNA-1323 downregulation promotes migration and invasion of breast cancer cells by targeting tumour protein D52. J Biochem. 2020;168:83–91.
12. Slotta-Huspenina J, Drecoll E, Feith M, Habermehl D, Combs S, Weichert W, et al. MicroRNA expression profiling for the prediction of resistance to neoadjuvant radiochemotherapy in squamous cell carcinoma of the esophagus. J Transl Med. 2018;16:109. Erratum in: J Transl Med. 2018;16:128.
13. Luo H, He J, Qin L, Chen Y, Chen L, Li R, et al. Mycoplasma pneumoniae lipids license TLR-4 for activation of NLPR3 inflammasome and autoagrophy to evoke a proinflammatory response. Clin Exp Immunol. 2020, http://dx.doi.org/10.1111/cei.13510. Epub ahead of print.
14. Shimizu T, Kida Y, Kuwano K. Mycoplasma pneumoniae-derived lipopeptides induce acute inflammatory responses in the lungs of mice. Infect Immun. 2008;76:270–7.
15. Liu F, Zhao Y, Lu J, Chen S, Zhang X, Mao W. Hyperside inhibits proinflammatory cytokines in human lung epithelial cells infected with Mycoplasma pneumoniae. Mol Cell Biochem. 2019;453:179–86.
16. Kazachkov MY, Hu PC, Carson J, Murphy PC, Henderson FW, Noah TL. Release of cytokines by human nasal epithelial cells and peripheral blood mononuclear cells infected with Mycoplasma pneumoniae. Exp Biol Med (Maywood). 2002;227:330–5.
17. Poddiegie D. Extra-pulmonary diseases related to Mycoplasma pneumoniae in children: recent insights into the pathogenesis. Curr Opin Rheumatol. 2018;30:380–7.
18. Yang Y, Yang F, Yu X, Wang B, Yang Y, Zhou H, et al. miR-16 inhibits NLPR3 inflammasome activation by directly targeting TLR4 in acute lung injury. Biomed Pharmacother. 2019;112:108664.
19. Li P, Yao Y, Ma Y, Chen Y. MIR-150 attenuates LPS-induced acute lung injury via targeting AKT3. Int Immunopharmacol. 2019;75:105794.
20. Kozloski GA, Jiang X, Bhatt S, Ruiz J, Vega F, Shakovitch R, et al. miR-181a negatively regulates NF-κB signaling and affects activated B-cell-like diffuse large B-cell lymphoma pathogenesis. Blood. 2016;127:2856–66.
21. Rady M, Watzl C, Claus M, Khorshid O, Mahran L, Abou-Aisha K. Altered expression of miR-181a and miR-146a does not change the expression of surface NCRs in human NK cells. Sci Rep. 2017;7:41381.

22. Fang J, Zhuge L, Rao H, Huang S, Jin L, Li J. Increased Levels of miR-155 are Related to Higher T-Cell Activation in the Peripheral Blood of Patients with Chronic Hepatitis B. Genet Test Mol Biomarkers. 2019;23:118-23.

23. Srinoun K, Nopparatana C, Wongchanchailert M, Fucharoen S. MiR-155 enhances phagocytic activity of β-thalassemia/HbE monocytes via targeting of BACH1. Int J Hematol. 2017;106:638-47.

24. Kumar M, Ahmad T, Sharma A, Mabalirajan U, Kulshreshtha A, Agrawal A, et al. Let-7 microRNA-mediated regulation of IL-13 and allergic airway inflammation. J Allergy Clin Immunol. 2011;128, 1077-85.e1-10.

25. Ying H, Kang Y, Zhang H, Zhao D, Xia J, Lu Z, et al. MiR-127 modulates macrophage polarization and promotes lung inflammation and injury by activating the JNK pathway. J Immunol. 2015;194:1239-51.

26. Hassan KS, Al-Khadouri G. Mycoplasma pneumoniae Pneumonia with Worsening Pleural Effusion Despite Treatment with Appropriate Antimicrobials: Case report. Sultan Qaboos Univ Med J. 2018;18:e239-42.

27. Cha SI, Shin KM, Jeon KN, Yoo SS, Lee J, Lee SY, et al. Clinical relevance and characteristics of pleural effusion in patients with Mycoplasma pneumoniae pneumonia. Scand J Infect Dis. 2012;44:793-7.

28. Yan C, Xue G, Zhao H, Feng Y, Li S, Cui J, et al. Molecular and clinical characteristics of severe Mycoplasma pneumoniae pneumonia in children. Pediatr Pulmonol. 2019;54:1012-21.

29. Yu J-S, Jin J, Li Y-y. The Physiological functions of IKK-selective substrate identification and their critical roles in diseases. STEMedicine. 2020;1:e49.

30. Kurai D, Nakagaki K, Wada H, Saraya T, Kamiya S, Fujioka Y, et al. Mycoplasma pneumoniae extract induces an IL-17-associated inflammatory reaction in murine lung: implication for mycoplasmal pneumonia. Inflammation. 2013;36:285-93.