Int J Clin Exp Med 2019;12(8):10101-10108
www.ijcem.com /ISSN:1940-5901/IJCEM0061503

Original Article

Downregulation of miR-19b in pediatric asthma is associated with the activation of nuclear factor-kappa B signaling pathway

Lizhong Ding[1], Chunjuan Li[2]

[1]*Department of Pediatric, Affiliated Hospital of Changchun University of Traditional Chinese Medicine, Changchun 130021, China;* [2]*Department of Gynecology, Nanguan District Hospital of Traditional Chinese Medicine Changchun City, Changchun 130041, China*

Received July 14, 2017; Accepted January 10, 2018; Epub August 15, 2019; Published August 30, 2019

Abstract: miR-19b is associated with asthma while nuclear factor-kappa B (NF-kappa B) signaling pathway plays an important role in asthma progression. Thus, miR-19b may affect asthma via NF-kappa B signaling pathway. All children were assigned into health group and asthma group. B cell line was transferred with miR-19b mimics and inhibitors. MiR-19b level and main molecules of NF-kappa B signaling pathway were measured by Real-time qRT-PCR and Western Blot. NF-kappa B immunoreactivity was measured by using anti-p65 NF-kappa B antibody. qRT-PCR showed that miR-19b level was higher in the health group than the asthma group ($P < 0.05$; post-test of Kruskal-Wallis and Dunn). Western Blot analyses demonstrated that the levels of phospho-p65 NF-kappa B, granulocyte-macrophage colony-stimulating factor and interleukin-8 were lowest in the health group and highest in the asthma group ($P < 0.05$). miR-19b overexpression reduced the levels of phospho-p65 NF-kappa B, GM-CSF and IL-8 when compared with the controls. In contrast, miR-19b inhibitor increased the levels of phospho-p65 NF-kappa B, GM-CSF and IL-8, when compared with the controls ($P < 0.05$). The percentage of B cells with p65 NF-kappa B staining was higher in the cells coinfected with the inhibitors (median, 25-75 percentiles: 49, 46-52) and wild type (median, 25-75 percentiles: 38, 25-36) than in the cells co-infected with the mimics (median, 25-75 percentiles: 8.5, 6-11) ($P < 0.05$). The results suggest miR-19b prevents the phosphorylation of p-65 NF-kappa B. miR-19b ameliorates pediatric asthma by suppressing NF-kappa B signaling, which activates inflammatory mediators. Serum miR-19b may be a potential drug for the therapy of pediatric asthma.

Keywords: Asthma, inflammation, nuclear factor-kappa B, granulocyte-macrophage colony-stimulating factor, interleukin-8

Introduction

Asthma is global burden of respiratory infections in children but the related molecular mechanisms remain widely unknown. The transcription factor nuclear factor-kappa B (NF-kappa B) regulates the expression of various inflammatory factors and can be activated by inflammatory stimuli [1]. NF-kappa B is a dimer consisting of subunits that include c-Rel, RelA (p65), RelB, p50 and p52 [2]. In most cells, the NF-kappa B prototype is a heterodimer consisting of p65 and p50, which is a subunit with transactivation function [3]. An en dogenous inhibitor Ikappa B regulates NF-kappa B activation by complexing with the transcription factor and capturing it in the cytoplasm [4]. Ikappa B molecules form different protein families, including IkappaBα, IkappaBβ, IkappaBε, IkappaBφ, IkappaBδ and p105 [5]. The most characteristic NF-kappa B inhibitor is IkappaBα [6]. This protein strongly binds to the p65 subunit of NF-kappa B by its ankyrin repeat domain associated with the nuclear localization signal and the immunoglobulin-like domain of p65 [7].

Activation of NF-kappa B results in increased expression of granulocyte-macrophage colony-stimulating factor (GM-CSF) [8] and IL-8 [9]. By inhibiting NF-kappa B activity, the expression of these cytokines is usually inhibited by

glucocorticoids (GC) [10, 11]. However, high-level GM-CSF and IL-8 were synthesized from airway cells and peripheral blood mononuclear cells (PBMC) in patients with severe asthma despite continuous long-term treatment of glucocorticoids [12]. These data indicate the continued activation of NF-kappa B in the patients with severe asthma [13, 14].

MicroRNAs (miRNAs) are small non-coding RNAs that fine-tune post-transcriptional gene expression by mRNA degradation or translation inhibition [15]. miR-19 is a major component of the miR-17~92 cluster, accelerating the production of Th2 cytokines, and amplifying the JAK-STAT signaling pathway to drive asthma pathogenic inflammation [16, 17]. NF-kappa B plays a key role in cytokine signaling [18]. MiR-19b expression reduces the levels of inflammatory cytokines [19], which may be caused by inhibiting phosphorylation of NF-kappa B. Important inflammatory cytokines such as IL-8 and GM-CSF can be activated by NF-kappa B. It appears that miR-19b may inhibit the production of multiple cytokines by down-regulating NF-kappa B, resulting in the reduction of asthma complications. Therefore, the effects of miR-19b on asthma were explored by investigating NF-kappa B signaling pathway.

Materials and methods

Patients

Sixty-eight patients with asthma were selected according to the American Thoracic Society [20]. The subjects with different-degree asthma: 28 patients with mild intermittent asthma, inhaled short-acting beta2 agonists (100 μg salmeterol daily, Hangzhou Dayangchem Co. Ltd., Hangzhou, China) if needed. Twenty-six patients with moderate asthma, inhaled short-acting beta2 agonists 100 μg salmeterol daily. Fourteen patients with severe uncontrolled asthma, took required high-dose GC (2,500 μg beclomethasone dipropionate (BDP, a kind of GC) daily, Zhejiang Xianju Xianle Pharmaceutical Co., Ltd., Taizhou, China), long-acting beta2 agonists (100 μg salmeterol) and oral prednisone (35 mg daily, Zhejiang Xianju Pharmaceutical Co. Ltd., Taizhou, China). No differences were found between health group and asthma group for age and gender ($P > 0.05$). The study was approved by the Ethics Committee of Affiliated Hospital of Changchun University of Traditional Chinese Medicine, and written informed consent was obtained from each patient.

Production of cytokines by PBMC

The PBMCs were isolated by Ficoll-Hypaque gradient centrifugation as previously described [21]. Freshly isolated PBMCs were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) medium containing 10% heat inactivated fetal bovine serum (FBS), 100 U penicillin ml^{-1}, 100 μg streptomycin ml^{-1} and 2 mM glutamine. In the absence or presence of BDP, salmeterol and oral prednisone, the cells were cultured for 3, 12 and 24 h. The supernatants were then harvested to measure the levels of phosphor-p65 NF-kappa B (ab176647), IL-8 (ab46032) and GM-CSF (ab100529) by using the ELISA kits from Abcam (Abcam Inc., Cambridge, MA, USA).

Cell coinfection

B cell lines were purchased from Shanghai cell bank (Shanghai, China). Antagomirs and mimics of miR-19b were synthesized by Sangon biotech co. ltd (Shanghai, China). The cell lines were coinfected with miR-19b antagomirs and mimics via Lipofectamine reagent (GenePharma Co., Shanghai, China). After three-day coinfection, the mutant cells were picked using 100 μg G-418 ml^{-1}. Phospho-p65 NF-kappa B, GM-CSF and IL-8 were measured in the cell lines by using ELISA kits.

Real-time PCR quantification of miR-19b

Total cellular RNA was isolated by using an RNA Isolation Total RNA Isolation Reagent (Shanghai Pufei Biotech Co., Ltd, 3101-100, Shanghai, China). cDNA was synthesized from total RNA by using a reverse transcription kit (TIANGEN, Beijing, China). The levels of miR-19b were measured by using real-time qPCR. MiR-19b, forward primer: 5'-GGGTGTGCAAATCCATGCA-3'; reverse primer: 5'-GTGCAGGGTCCGAGGT-3'. U6, forward primer: 5'-TTGGTGCTCGCTTCGGCA-3'; reverse primer: 5'-GTGCAGGGTCCGAGGT-3'. U6 snRNA transcripts were used as internal controls for miRNA expression.

Western blot

The antibodies for Phospho-p65 NF-kappa B (ab86299), GM-CSF (ab14024), IL-8 (ab118-

Table 1. Clinical characteristics of the subjects in present study

Characteristic	Normal	Asthma		
		Mild Controlled	Moderate Uncontrolled	Severe Uncontrolled
Number	30	28	26	14
Age, years*	8 (4-14)	9 (5-14)	8 (4-14)	9 (5-14)
male/female	21/9	20/8	19/7	10/4
Duration of asthma, years*	NA	5.2 (2.8-10.6)	6.7 (3.4-11.2)	7.5 (4.5-12.3)
FEV, % predicted*	100 (100-102)	82.3 (81.4-87.5)	56.8 (53.2-62.8)	51.7 (48.5-67.2)
Oral prednisone dose, mg/day*	0	0	0	35 (25-60)
Inhaled GC dose, equivalent BDP mg/day*	0	0	1,100 (1,000-1,400)	2,500

*Results were presented as medians with 25-75 percentiles shown in parentheses. FEV, Forced expiratory ratio; BDP, beclomethasone dipropionate; GC, glucocorticoid; NA, not applicable.

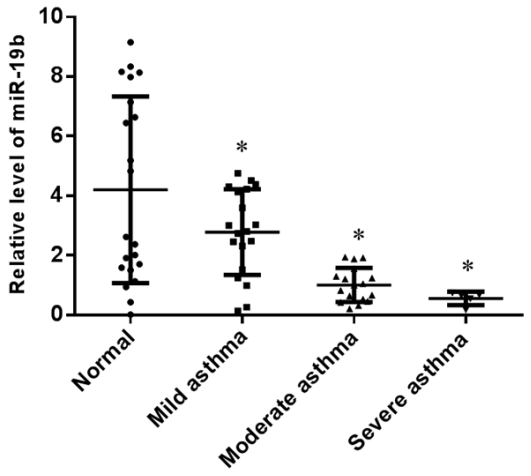

Figure 1. Relative levels of miR-19b in different groups. N = 30, 28, 26 and 14 cases in normal, mild asthma, moderate asthma and severe asthma groups, respectively. *P < 0.05 via a health group.

617), GAPDH (ab8245) and goat anti-rabbit HRP (ab205718) were purchased from Abcam. Cell extracts from B cells were prepared as described previously [22]. Forty-microgram total protein was subjected to SDS-PAGE on a 6 to 10% gradient gel and blotted onto a nitrocellulose membrane (Millipore, St Charles, MO, USA). The membrane was blocked with phosphate buffered saline (PBS) containing 3% bovine serum albumin and 0.1% Tween 20, and then cultured with primary antibody. After continuous washing with PBS containing 0.1% Tween 20, the membrane was incubated with a peroxidase conjugated secondary antibody. The protein brands were enlightened using an enhanced chemiluminescence system (Ambion, Austin, TX, USA) followed by autoradiography. The film was analyzed NIH image analysis software (NIH, Bethesda, MD, USA).

Immunocytochemistry analysis

B cells with different treatments were collected by centrifuge and fixed in cold acetone-chloroform (1:1). NF-kappa B immunoreactivity was measured by using an anti-p65 NF-kappa B antibody (Cat no. ab16502, Abcam). Relative protein levels were measured by using labeled streptavidin-biotin kit system (BIOSS, Beijing, China). p65 localization was expressed as the percentage of B cells showing a p65 nuclear staining.

Statistical analysis

Kruskal-Wallis and the Dunn's post tests were used for between-group comparisons. Mann-Whitney test was used for unpaired comparisons. Wilcoxon test was used for paired comparisons. Correlations were analyzed by Spearman rank test. Statistical significance was set at P < 0.05.

Results

Baseline characteristics of subjects

The demographic characteristics of normal subjects, mildly controlled, moderately uncontrolled, severely uncontrolled asthma children were shown in **Table 1**. Severe uncontrolled asthma patients had similar age with other children (P > 0.05; P < 0.05, Wilcoxon test). The children with severe uncontrolled asthma had a longer duration, and were significantly different from other subjects (P < 0.05; Wilcoxon test). Despite chronic GC therapy, severe uncontrolled airway injury in asthma children remains high and higher than moderate asthma children (P < 0.05; Wilcoxon test). In addition, all moderate asthma patients responded to oral GC trials because they had

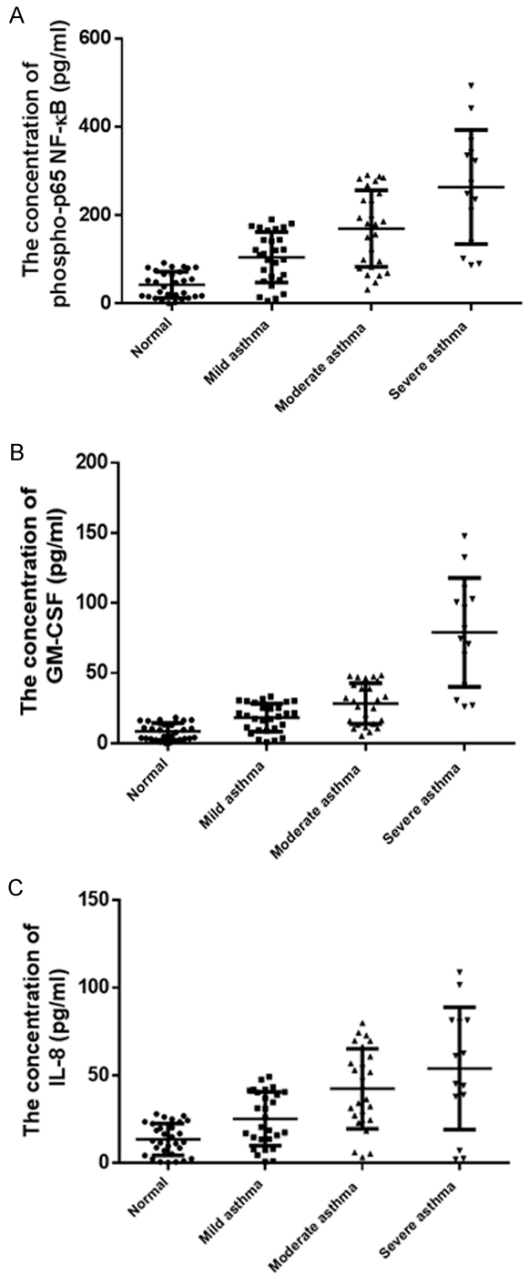

Figure 2. The serum concentrations of phospho-p65 NF-kappa B, GM-CSF and IL-8 in different groups. A. The serum concentration of phospho-p65 NF-kappa B in different groups. B. The serum concentration of GM-CSF in different groups. C. The serum concentration of IL-8 in different groups. All the concentrations were measured by ELISA kits. *$P < 0.05$ *vs.* a health group.

improved FEV (Forced expiratory ratio, $P = 0.006$; Wilcoxon test).

Serum levels of miR-19b in asthma patients

Among four different groups, the levels of miR-19b were highest in a health group. The serum levels of miR-19b were reduced with the asthma progression and reached the lowest level in a severe asthma group (**Figure 1**, $P < 0.05$; post-test of Kruskal-Wallis and Dunn). The results suggest that low-level of miR-19b may be associated with asthma development.

The levels of phospho-p65 NF-kappa B, GM-CSF and IL-8 in different groups

Among four different groups, the levels of phospho-p65 NF-kappa B were lowest in a health group. The serum levels of phospho-p65 NF-kappa B were increased with the asthma progression and reached the highest level in a severe asthma group ($P < 0.05$, **Figure 2A**). The results suggest that high-level of phospho-p65 NF-kappa B may be associated with asthma development. Similarly, among four different groups, the levels of GM-CSF and IL-8 were lowest in a health group. The serum levels of GM-CSF and IL-8 were increased with the asthma progression and reached the highest level in a severe asthma group (**Figure 2B** and **2C**, $P < 0.05$; post-test of Kruskal-Wallis and Dunn). The results suggest that high-level of cytokines GM-CSF and IL-8 may be associated with asthma development.

The effects of BDP, salmeterol and prednisone on PBMCs

The levels of miR-19b were stable in the presence or absence of BDP, salmeterol and prednisone (**Figure 3A**). In contrast, BDP, salmeterol and prednisone reduced the levels of phospho-p65 NF-kappa B (**Figure 3B**), GM-CSF (**Figure 3C**) and IL-8 (**Figure 3D**) when compared with control groups ($P < 0.05$; Mann-Whitney test) (**Figure 3**). The results suggest that all the medicine treatment inhibits the release of phosphor-p65 NF-kappa B, GM-CSF and IL-8 significantly but not miR-19b.

Western blot

Figure 4A showed the relative levels of miR-19b in different groups (CG, control group; SG, miR-19b was silenced by antagomirs. OG, miR-19b was overexpressed). The level was highest in OG and Lowest in SG. To measure the effects of miR-19b on the changes of phospho-p65 NF-kappa B, and phospho-p38 MAPK, Western Blot analysis was performed. Western Blot analysis shows that miR-19b overexpression reduces the levels of phos-

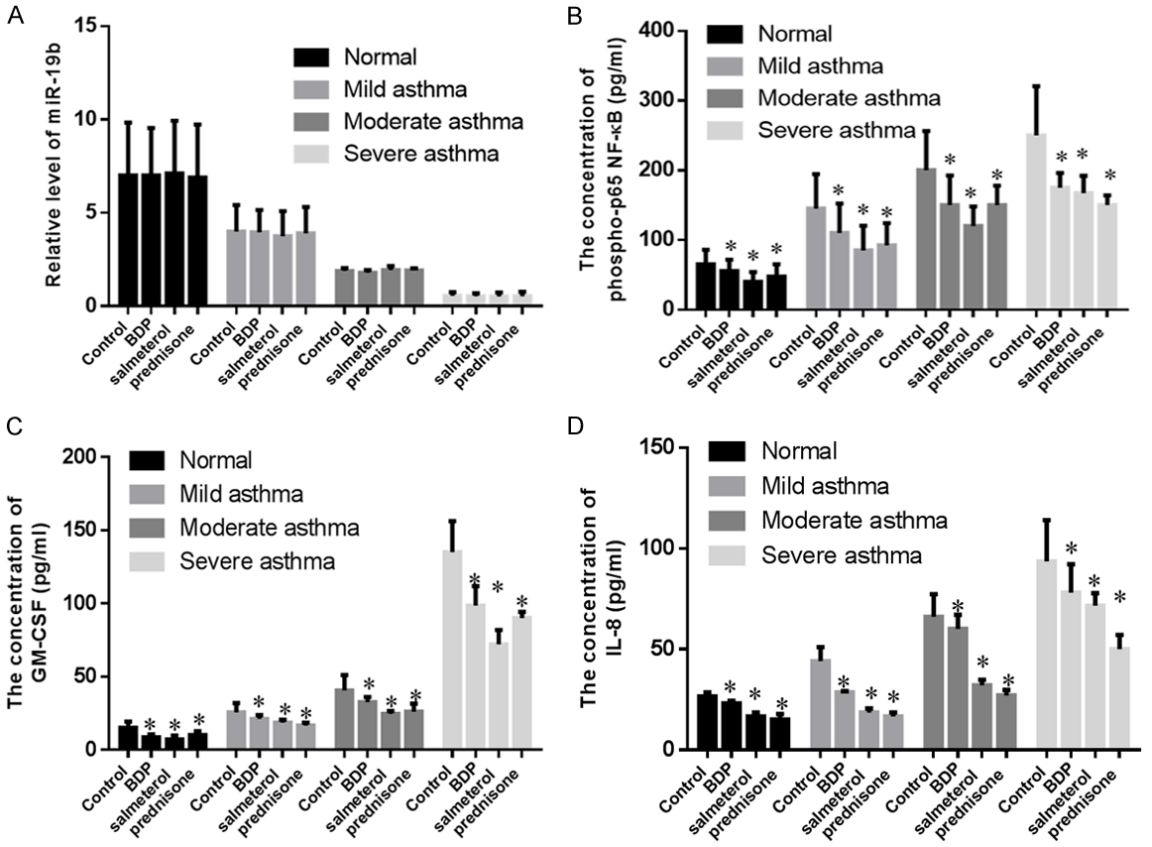

Figure 3. The effects of BDP, salmeterol and prednisone on PBMCs. A. The effects of BDP, salmeterol and prednisone on relative level of miR-19b. B. The effects of BDP, salmeterol and prednisone on the concentration of phosphor-p65 NF-kappa B. C. The effects of BDP, salmeterol and prednisone on the concentration of GM-CSF. D. The effects of BDP, salmeterol and prednisone on the concentration of IL-8. *$P < 0.05$ vs. a control group without BDP, salmeterol and prednisone treatment.

pho-p65 NF-kappa B (**Figure 4B** and **4C**), GM-CSF (**Figure 4B** and **4D**) and IL-8 (**Figure 4B** and **4E**) when compared with the level of control groups. In contrast, miR-19b inhibitor increases the levels of phospho-p65 NF-kappa B (**Figure 4C**), GM-CSF (**Figure 4D**) and IL-8 (**Figure 4E**), when compared with the level of control groups ($P < 0.05$; post-test of Kruskal-Wallis and Dunn). All the results suggest that miR-19b can affect the activity of NF-kappa B signaling.

Subcellular distribution of phospho-p65 NF-kappa B subunit

To determine the effects of miR-19b on subcellular distribution of p65 NF-kappa B subunit, we performed immunocytochemistry analysis in B cell line infected with miR-19b mimics or inhibitors. Immunostaining showed that the percentage of B cells with a p65 NF-kappa B staining was higher in the cells coinfected with the inhibitors (median, 25-75 percentiles: 49, 46-52) and wild type (median, 25-75 percentiles: 38, 25-36) than in the cells coinfected with the mimics (median, 25-75 percentiles: 8.5, 6-11) ($P < 0.05$) (**Figure 5**, $P < 0.05$; Spearman rank test). The results suggest miR-19b prevents the phosphorylation of p-65 NF-kappa B.

Discussion

Despite the use of GC for asthma therapy, subjects with severe uncontrolled asthma exhibit persistent airway inflammation characterized by activated neutrophils, T lymphocytes and eosinophils [23]. This inflammation may continue to be increased by the cytokines such as GM-CSF and IL-8, not only in airway cells but also in PBMCs isolated from subjects with severe uncontrolled asthma. We have shown that PBMCs from the subjects with severe uncontrolled asthma can release these inflam-

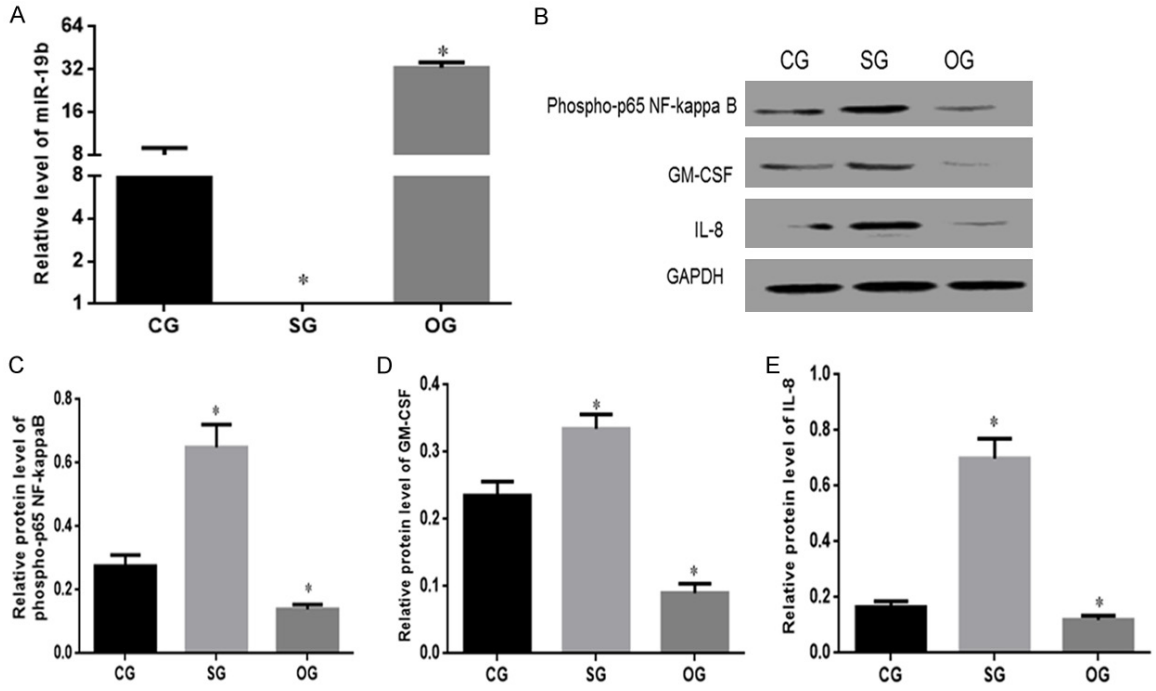

Figure 4. The effects of miR-19b on the expression of NF-kappa B signaling molecules. A. The levels of miR-19b in different groups. B. Western Blot analysis of the effects of miR-19b on the expression of NF-kappa B signaling molecules. C. The relative protein level of phospho-p65 NF-kappa B in different groups. D. The relative protein level of GM-CSF in different groups. E. The relative protein level of IL-8 in different groups. CG, control group. SG, miR-19b was silenced by antagomirs. OG, miR-19b was overexpressed. **P* < 0.05 vs. a CG group.

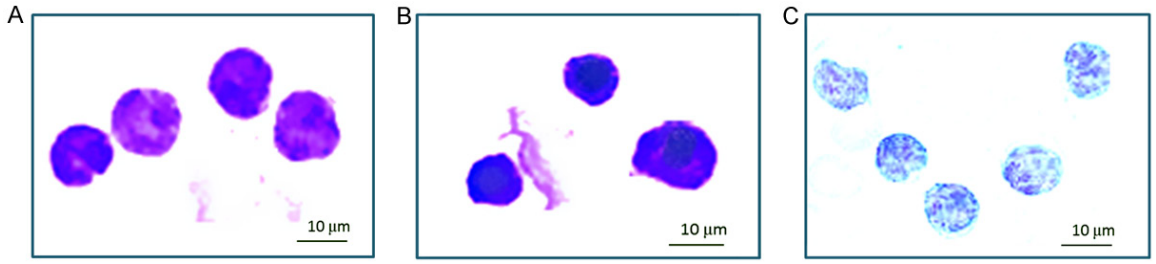

Figure 5. The subcellular distribution of phosphor-p65 nuclear factor kappa B (NF-kappa B) subunit by immunocytochemistry. A. p65 is located in the cytosol of B cells of wild type; B. p65 is located in the cytosol and nuclei of B cells infected with miR-19b inhibitors; C. p65 is located in the cytosol of B cells infected with miR-19b mimics.

matory cytokines due to abnormal expression of NF-kappa B, suggesting that other mechanisms may be associated inflammation responses.

Although the biological and clinical relevance of these results requires further support from greater studies, these data extend previous observations that p65 expression increases in bronchial epithelial cells in patients with severe GC-dependent asthma [24]. Importantly, p65 overexpression increases NF-kappa B transcriptional activity, as shown in the transfection study. The continued activation of the NF-kappa B system appears to be associated with poorly controlled disease of moderate and severe uncontrolled asthma. There may be excessive activity of NF-kappa B receptor in severely uncontrolled asthma patients.

PBMCs have high-level miR-19b in a health group, suggesting that miR-19b may control asthma by reducing inflammatory microenvironment via the inhibition of NF-kappa B. In addition, sustained NF-kappa B activation in severe asthma patients was also demonstrat-

ed by increased p65 DNA binding and nuclear p65 localization. Although it is believed that the sustained activation of the NF-kappa B system in patients with severe asthma is undesirable. The correlation between low-level serum miR-19b and high-level NF-kappa B level also supports this problem.

The present results show that serum miR-19b can prevent pediatric asthma by inhibiting NF-kappa B signaling pathway, which is associated with asthma development. Serum miR-19b may be a potential drug for the therapy of pediatric asthma. To confirm that, further work is needed to be done in the future.

Disclosure of conflict of interest

None.

Address correspondence to: Lizhong Ding, Department of Pediatric, Affiliated Hospital of Changchun University of Traditional Chinese Medicine, Workers and Peasants Road 1478, Changchun 130021, China. Tel: +86-0431-88751449; Fax: +86-0431-88751449; E-mail: dinglzmm@163.com

References

[1] Levrand S, Pesse B, Feihl F, Waeber B, Pacher P, Rolli J, Schaller MD and Liaudet L. Peroxynitrite is a potent inhibitor of NF-{kappa}B activation triggered by inflammatory stimuli in cardiac and endothelial cell lines. J Biol Chem 2005; 280: 34878-34887.

[2] Dai R, Phillips RA and Ahmed SA. Despite inhibition of nuclear localization of NF-kappa B p65, c-Rel, and RelB, 17-beta estradiol up-regulates NF-kappa B signaling in mouse splenocytes: the potential role of Bcl-3. J Immunol 2007; 179: 1776-1783.

[3] Pascal V, Nathan NR, Claudio E, Siebenlist U and Anderson SK. NF-kappa B p50/p65 affects the frequency of Ly49 gene expression by NK cells. J Immunol 2007; 179: 1751-1759.

[4] Moorthy AK and Ghosh G. p105.Ikappa Bgamma and prototypical Ikappa Bs use a similar mechanism to bind but a different mechanism to regulate the subcellular localization of NF-kappa B. J Biol Chem 2003; 278: 556-566.

[5] Jin H, Yan Z, Ma Y, Cao Y and He B. A herpesvirus virulence factor inhibits dendritic cell maturation through protein phosphatase 1 and Ikappa B kinase. J Virol 2011; 85: 3397-3407.

[6] Nomura A, Majumder K, Giri B, Dauer P, Dudeja V, Roy S, Banerjee S and Saluja AK. Inhibition of NF-kappa B pathway leads to deregulation of epithelial-mesenchymal transition and neural invasion in pancreatic cancer. Lab Invest 2016; 96: 1268-1278.

[7] Gueguen G, Kalamarz ME, Ramroop J, Uribe J and Govind S. Polydnaviral ankyrin proteins aid parasitic wasp survival by coordinate and selective inhibition of hematopoietic and immune NF-kappa B signaling in insect hosts. PLoS Pathog 2013; 9: e1003580.

[8] Sundaram K, Mitra S, Gavrilin MA and Wewers MD. House dust mite allergens and the induction of monocyte interleukin 1beta production that triggers an IkappaBzeta-dependent granulocyte macrophage colony-stimulating factor release from human lung epithelial cells. Am J Respir Cell Mol Biol 2015; 53: 400-411.

[9] Chan LP, Liu C, Chiang FY, Wang LF, Lee KW, Chen WT, Kuo PL and Liang CH. IL-8 promotes inflammatory mediators and stimulates activation of p38 MAPK/ERK-NF-kappaB pathway and reduction of JNK in HNSCC. Oncotarget 2017; 8: 56375-56388.

[10] Fei X, Zhang PY, Zhang X, Zhang GQ, Bao WP, Zhang YY, Zhang M and Zhou X. IL-17A monoclonal antibody partly reverses the glucocorticoids insensitivity in mice exposed to Ozonec. Inflammation 2017; 40: 788-797.

[11] Fry CS, Nayeem SZ, Dillon EL, Sarkar PS, Tumurbaatar B, Urban RJ, Wright TJ, Sheffield-Moore M, Tilton RG and Choudhary S. Glucocorticoids increase skeletal muscle NF-kappaB inducing kinase (NIK): links to muscle atrophy. Physiol Rep 2016; 4.

[12] Zhang N, Truong-Tran QA, Tancowny B, Harris KE and Schleimer RP. Glucocorticoids enhance or spare innate immunity: effects in airway epithelium are mediated by CCAAT/enhancer binding proteins. J Immunol 2007; 179: 578-589.

[13] Bui TT, Piao CH, Kim SM, Song CH, Shin HS, Lee CH and Chai OH. Citrus tachibana leaves ethanol extract alleviates airway inflammation by the modulation of Th1/Th2 imbalance via inhibiting NF-kappaB signaling and histamine secretion in a mouse model of allergic asthma. J Med Food 2017.

[14] Jiao D, Wong CK, Tsang MS, Chu IM, Liu D, Zhu J, Chu M and Lam CW. Activation of eosinophils interacting with bronchial epithelial cells by antimicrobial peptide LL-37: implications in allergic asthma. Sci Rep 2017; 7: 1848.

[15] Sims EK, Lakhter AJ, Anderson-Baucum E, Kono T, Tong X and Evans-Molina C. MicroRNA 21 targets BCL2 mRNA to increase apoptosis in rat and human beta cells. Diabetologia 2017; 60: 1057-1065.

[16] Levänen B, Bhakta NR, Torregrosa Paredes P, Barbeau R, Hiltbrunner S, Pollack JL, Sköld

CM, Svartengren M, Grunewald J, Gabrielsson S, Eklund A, Larsson BM, Woodruff PG, Erle DJ, Wheelock ÅM. Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients. J Allergy Clin Immunol 2013; 131: 894-903.

[17] Simpson LJ, Patel S, Bhakta NR, Choy DF, Brightbill HD, Ren X, Wang Y, Pua HH, Baumjohann D and Montoya MM. A microRNA upregulated in asthma airway T cells promotes TH2 cytokine production. Nature Immunology 2014; 15: 1162-1170.

[18] Hayashi T, Kaneda T, Toyama Y, Kumegawa M and Hakeda Y. Regulation of receptor activator of NF-kappa B ligand-induced osteoclastogenesis by endogenous interferon-beta (INF-beta) and suppressors of cytokine signaling (SOCS). The possible counteracting role of SOCSs- in IFN-beta-inhibited osteoclast formation. J Biol Chem 2002; 277: 27880-27886.

[19] Cheng X, Zhang X, Su J, Zhang Y, Zhou W, Zhou J, Wang C, Liang H, Chen X, Shi R, Zen K, Zhang CY and Zhang H. miR-19b downregulates intestinal SOCS3 to reduce intestinal inflammation in Crohn's disease. Sci Rep 2015; 5: 10397.

[20] Tarlo SM, Malo JL, de Blay F, Le Moual N, Henneberger P, Heederik D, Raulf M, Carlsten C, Cartier A; ATS Assembly on Environmental, Occupational, and Population Health. An official american thoracic society workshop report: presentations and discussion of the sixth Jack Pepys workshop on asthma in the workplace. Ann Am Thorac Soc 2017; 14: 1361-1372.

[21] Ulmer A, Scholz W, Ernst M, Brandt E and Flad HD. Isolation and subfractionation of human peripheral blood mononuclear cells (PBMC) by density gradient centrifugation on Percoll. Immunobiology 1984; 166: 238-250.

[22] Rühl R. Method to determine 4-oxo-retinoic acids, retinoic acids and retinol in serum and cell extracts by liquid chromatography/diode-array detection atmospheric pressure chemical ionisation tandem mass spectrometry. Rapid Commun Mass Spectrom 2006; 20: 2497-2504.

[23] Halwani R, Sultana Shaik A, Ratemi E, Afzal S, Kenana R, Al-Muhsen S and Al Faraj A. A novel anti-IL4Ralpha nanoparticle efficiently controls lung inflammation during asthma. Exp Mol Med 2016; 48: e262.

[24] Vignola AM, Chiappara G, Siena L, Bruno A, Gagliardo R, Merendino AM, Polla BS, Arrigo AP, Bonsignore G, Bousquet J and Chanez P. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. J Allergy Clin Immunol 2001; 108: 738-746.