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To cite this article: Hong Zhang, Yuanchun Sun, Wei Rong, Linxia Fan, Yufeng Cai, Qiang Qu, Yun Gao & Hongxia Zhao (2019): miR-221 participates in the airway epithelial cells injury in asthma via targeting SIRT1, *Experimental Lung Research*, DOI: [10.1080/01902148.2018.1533051](https://doi.org/10.1080/01902148.2018.1533051)

To link to this article: <https://doi.org/10.1080/01902148.2018.1533051>



Published online: 17 Jan 2019.



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ORIGINAL ARTICLE



miR-221 participates in the airway epithelial cells injury in asthma via targeting SIRT1

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ABSTRACT

Aim of the Study: To investigate the role of microRNA-221 (miR-221) in the airway epithelial cell injury in asthma and delineate the underlying mechanism that may involve with SIRT1. **Materials and Method:** Bronchial epithelial cells from asthma patients and healthy controls were obtained by bronchoscopic brushing. The miR-221 and SIRT1 mRNA level in collected cells were detected by qRT-PCR. BEAS2B cell lines were cultured *in vitro*. In order to up-regulate miR-221 and SIRT1, miR-221 mimic and pcDNA3.1-SIRT1 vector was transfected into BEAS2B cells, respectively. The expression changes of miR-221 and SIRT1 after transfection was observed by qRT-PCR and Western blot. The target relationship between miR-221 and SIRT1 was confirmed using dual-luciferase reporter assay. The cell viability changes after transfection was measured using cellTiter-blue reagent. The apoptosis rate was detected by flow cytometry. **Result:** Compared with healthy controls, miR-221 expression significantly increased in bronchial epithelial cells from patients subjects. In contrast, the level of SIRT1 mRNA reduced in the bronchial epithelial cell from asthma patients. *In vitro*, up-regulation of miR-221 could inhibit the expression of SIRT1 both at mRNA and protein level in BEAS2B cells. A negative correlation between miR-221 and SIRT1 mRNA in samples from patients was confirmed and dual-luciferase reporter assay showed that miR-221 directly binds to the 3'UTR of SIRT1 mRNA. Overexpression of miR-221 or SIRT1 knockdown could inhibit proliferation but induce apoptosis in BEAS2B cells. Moreover, up-regulation of SIRT1 could antagonize miR-221's inhibitory effect. **Conclusion:** miR-221 may participate in the airway epithelial cells injury in asthma via targeting SIRT1.

ARTICLE HISTORY

Received 9 April 2018
Accepted 2 October 2018

KEYWORDS

Asthma; microRNA;
microRNA-221; SIRT1;
Bronchial epithelial cells

Background

Asthma is chronic, inflammatory condition of the lower airways characterized by reversible airflow obstruction, airway hyper-responsiveness (AHR) and remodeling of airway tissues.¹ Increasing experimental evidence suggests that airway epithelium plays a pivotal role in asthma pathogenesis.² Airway epithelial cells recognized as the first line of defense against exposure of airway tissue to an inflammatory reaction. Airway epithelial cells injury is one of the characteristics of asthma, significantly associated with AHR, mucus overproduction, allergic cascade and airway inflammation.^{3,4} Previous *vivo* and *vitro*

experiments have identified that inflammatory cytokines and reactive oxygen species induced by oxidative stress are essential inducers of airway epithelial cells damage.⁵ For instance, Gurpreet et al, showed that, IL-9 and IL-13, which are Th2 cells related cytokines, lead to dysregulation of airway epithelial cell death and epithelial repair in asthma. Given that airway epithelial cells injury immensely contributes to asthma pathophysiology, maintaining airway epithelial cell survival and function may be an effective therapeutic strategy for asthma.⁶ However, the details of the molecular mechanism of airway epithelial cell injury in asthma are still under active research.

microRNAs are approximately 22nt short endogenous non-coding RNAs that regulate post-transcriptional gene expression. By binding to the 3' untranslated region (3'-UTR) of target genes, microRNAs could induce the mRNA degradation or translation inhibition of their target genes.⁷ An astonishing number of connections between microRNAs and diseases have been extensively investigated due to their crucial role in biological processes including cell proliferation and apoptosis. microRNAs also have been implicated in the development of asthma; microRNAs not only provide novel biomarkers but also therapeutic strategies in asthma.^{8,9} Recently, studies highlighted that microRNA-221 (miR-221) regulates the pathogenesis of asthma. Liu et al showed that miR-221 up-regulated in pediatric asthma.¹⁰ Qin suggested that inhibition of miR-221 suppressed the airway inflammation in asthma.¹¹ But whether or not miR-221 mediated the airway epithelial cells injury in asthma have not been thoroughly investigated.

SIRT1, also known as NAD-dependent deacetylase sirtuin-1, is an enzyme that deacetylates proteins that contribute to cellular regulation. SIRT1 play a role in cell growth, cell differentiation, cell aging, energy metabolism, and anti-inflammation.¹² Most importantly, studies showed that SIRT1 is associated with cell survival and often down-regulated in cell injury. For example, Bean et al. found a SIRT1 pathway dysregulation in smoke-exposure airway epithelial cells. Induction of activity of SIRT1 could protect airway epithelial cells from injury.¹³ Wang et al., propose that decreasing SIRT1 levels in lung tissue represent a new biological characteristic of asthma. Bioinformatical data indicated that miR-221 may target on SIRT1.¹⁴ Based on this evidence, we provided a hypothesis that miR-221 may be involved with the injury of airway epithelial cells in asthma via targeting SIRT1.

Materials and method

Kit and reagent

Lipofectamine[®] RNAimax and Lipofectamine[®] 2000 reagent was purchased from Thermo Fisher Scientific (USA). microRNA-221 mimics and siRNA-SIRT1 were purchased from Shanghai

genepharma (China). Trizol reagent and SYBR Green qPCR Master Mix kit were purchased from Thermo Fisher Scientific (USA). miScript II RT Kit and miScript SYBR[®] Green PCR Kit were purchased from Qiagen (USA). All primers design and were purchased from Shanghai genepharma. SIRT1, GAPDH antibodies and HRP labeled Anti-mouse IgG antibodies purchased from Santa Cruz Biotechnology (USA). CellTiter-Blue[®] Cell Viability Assay KIT and Luciferase Assay System were purchased from Promega (USA). Annexin-PI apoptosis analysis kit was purchased from Beyotime Biotechnology (China).

Bronchial epithelial cells collection

Bronchial epithelial cells from asthma patients (15 males and 10 females, average 51 years old) were obtained by bronchoscopic brushing. All asthma patients were diagnosed as severe asthma according to the define from Global Initiative for Asthma 2016 (GINA2016) and undergone inhaled corticosteroid treatment. Asthma severity was evaluated by a respiratory physician based on a combination of symptoms, lung function, and amount of medication. The exclusion criteria for asthma patients were: a history of cigarette smoking, pregnancy, acute or chronic upper respiratory tract infection and other condition which were not unsuitable for enrollment in the present study. As a control, bronchial cells were collected from healthy volunteers using the same technique. Healthy volunteers must have no history of asthma/allergic rhinitis and selected with the above-mentioned exclusion criteria. All subjects' demographic characteristics was shown in Table 1. This collection protocol was approved by the *Ethics Committee of the people's Hospital of Gansu Province* and all patients and volunteer provided their informed consents.

Table 1. Demographic characteristics of the Healthy and asthmatic subjects.

	Healthy control	Patients with asthma
Sex (Male/Female)	10/12	15/10
Average Age	53 ± 6	51 ± 7
FEV ₁ (%)	97.8 ± 8.5	55.6 ± 6.8
FEV ₁ /FVC ration (%)	79.2 ± 8.6	63.2 ± 7.6
Median ICS dose (µg/d)	N/A	2100 (1500 ~ 3500)

Cell culture and transfection

Bronchial epithelial cells collected from patients and volunteers were seeded into 1% collagen coated dishes and cultured in LHC-9 media. For *in vitro* experiments, BEAS2B cells were purchased from American Type Culture Collection (ATCC) and cultured in keratinocyte media supplemented with human recombinant epithelial growth factor (EGF) and bovine pituitary extracts (BPE). All cells were maintained at 37 °C 5%CO₂ in a humidified incubator.

For up-regulating miR-221 and SIRT1 knock-down, miR-221 mimic and SIRT1 siRNA was transfected into BEAS2B cells using Lipofectamine[®] RNAimax kit, respectively. A validated random sequence (no identifiable effects on known miRNA function) was also transfected as a transfection negative control (transfection-NG). For overexpression SIRT1, pcDNA3.1-SIRT1 eukaryotic expressing plasmid was transfected into BEAS2B cells using Lipofectamine[®] 2000 reagent. Empty pcDNA3.1 vector was used as negative control.

Real-Time quantitative PCR (qRT-PCR)

Total RNA samples were extracted from cells using Trizol reagent. For detection of miR-221, RNA was reverse-transcribed using miScript II RT Kit then obtained cDNA was amplified and quantified using miScript SYBR[®] Green PCR Kit. RNU 48 was detected as the endogenous control for data normalization. For SIRT1 mRNA quantifying, cDNA was synthesized using PrimeScript[™] RT Master Mix and detected by SYBR Green qPCR Master Mix kit. GAPDH was used as reference gene. After the reactions, the relative levels of target genes were calculated by 2^{-(ΔΔCT)} method.

Western blot

Total protein samples were isolated from cells using RIPA reagent containing protease inhibitor cocktail. protein samples were quantified using BSA assay and heat up with loading buffer at 100 °C for 10 min. 50 μg proteins were separated through in a 12% SDS-PAGE gel at 100 V for 1.5h and then transferred to nitrocellulose

membranes. After blocked by 5% milk, membranes were incubated with mouse anti-SIRT1 antibodies or mouse anti-GAPDH (as reference) and followed with HRP labeled Anti-mouse IgG antibodies incubation. Protein bands were detected by ECL chemiluminescence Kit.

Cell viability assay

Transfected BEAS2B cells were planted into 96-well plate at the density of 3000 cells/well. Cell viability was measured by CellTiter-Blue[®] Cell Viability Assay KIT at 24 h, 48 h, 72 h, and 96 h after cultured. The viability relative to 24 h was calculated by the formula: $24\text{ h relative cell viability} = \text{cell viability [another time point]} / \text{cell viability [24 h]}$.

Apoptosis assay

Transfected BEAS2B cells were cultured 78 h and collected. After washing with PBS, cells stained with Annexin V-FITC and propidium iodide (PI) for 30 min in dark room. Flow cytometer was used to detect the apoptosis rate of cells. The data were analyzed by Flow Jo software

Dual-Luciferase reporter assay

Luciferase Assay System was used to confirm the target relationship between miR-221 and SIRT1 mRNA. Briefly, wildtype 3'-UTR sequence of SIRT1 containing miR-221 binding site and mutant 3'-UTR sequence of SIRT1 was inserted into PsiCHECK2 luciferase reporter vectors (PsiCHECK2 -SIRT1 3'-UTR-Wt and PsiCHECK2-SIRT13'-UTR-Mut). These PsiCHECK2 vectors were co-transfected with miR-221 mimic or mimic negative control into BEAS2B cells, respectively. The cells were harvested and relative luciferase activity (activity firefly/activity renilla) was detected.

Statistical analysis

The data statistical analysis was processed with R Statistical Software (3.4.1). Data were expressed as Mean ± standard deviation ($\bar{x} \pm SD$). The statistical significance between two groups was

analyzed by t-test. The statistical significance among more than two groups was accessed by one-way analysis of variance followed by LSD-t-test. A $P < 0.05$ probability level was considered to be statistically significant.

Results

miR-221 and SIRT1 expression changes in bronchial epithelial cells from asthma patients

miR-221 and SIRT1 expression in bronchial epithelial cells collected from bronchial epithelial brushings from asthma patients and healthy controls were examined using qRT-PCR. The level of miR-221 and SIRT1 mRNA were altered in samples from patient subjects. As shown in Figure 1A, the miR-221 was confirmed to be significantly up-regulated in bronchial epithelial cells from patients. In the other hand, the mRNA level of SIRT1 was statistical significantly down-regulated in patients with asthma compared to healthy controls (Figure 1B). Correlation analysis showed a significant negative correlation ($r = 0.68$, $P < 0.05$) between miR-221 and SIRT1 mRNA among the samples from patients (Figure 1C). This evidence indicated a possible trend towards higher miR-221 level with lower SIRT1 mRNA level in bronchial epithelial cells under asthmatic condition.

miR-221 directly regulates the expression of SIRT1 in BEAS2B cells

To confirm the whether miR-221 could regulate the expression of SIRT1 in bronchial epithelial cells we conducted *in vitro* experiments. The

BEAS2B cells were transfected with miR-221 mimics in order to up-regulate miR-221 (Figure 2A). As shown in Figure 2B and C, overexpression miR-221 could reduce the mRNA level and protein level of SIRT1 in BEAS2B. In dual-luciferase reporter assay, a lower luciferase activity was only observed in cells co-transfected with miR-221 mimics and 3'-UTR-Wt reporter plasmid (Figure 2D). These results indicated that miR-221 directly target on SIRT1 in BEAS2B cells by binding SIRT1's 3'UTR.

miR-221 may modulate the proliferation and apoptosis of BEAS2B cells by targeting SIRT1

To access the role of miR-221 overexpression in the bronchial epithelial cells, the proliferation and apoptosis of miR-221 mimic transfected BEAS2B cells were measured. As shown in Figure 3, up-regulation of miR-221 and SIRT1 knockdown significantly inhibited the cell proliferation but induced apoptosis. To reveal the potential mechanisms by which miR-221 has an inhibitory effect on BEAS2B cells, a functional rescue experiment was designed. We synchronized SIRT1 up-regulating with miR-221 mimic transfection. Over-expression of SIRT1 partly rescued the inhibition of proliferation caused by miR-221 and also reduced apoptosis (Figure 3). In our preliminary experiment, we did not observe any effect of only up-regulating SIRT1 or miR-221 inhibition on cells' proliferation and apoptosis (data not shown). These data suggested that miR-221 may have an inhibitory effect on BEAS2B cells via targeting SIRT1.

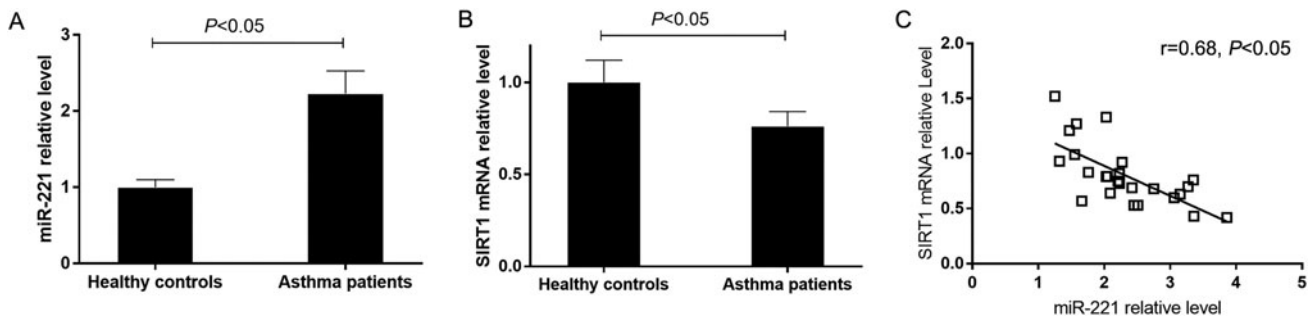


Figure 1. The expression difference of miR-221 and SIRT1 in bronchial epithelial cells between healthy and patients with asthma. miR-221 increased in patients bronchial epithelial cells (A), meanwhile, the SIRT1 levels decreased (B). A negative correlation between miR-221 and SIRT1 level in bronchial epithelial cells collected from patients were observed (C). Data represent Mean $\pm$ SD, $n = 3$.

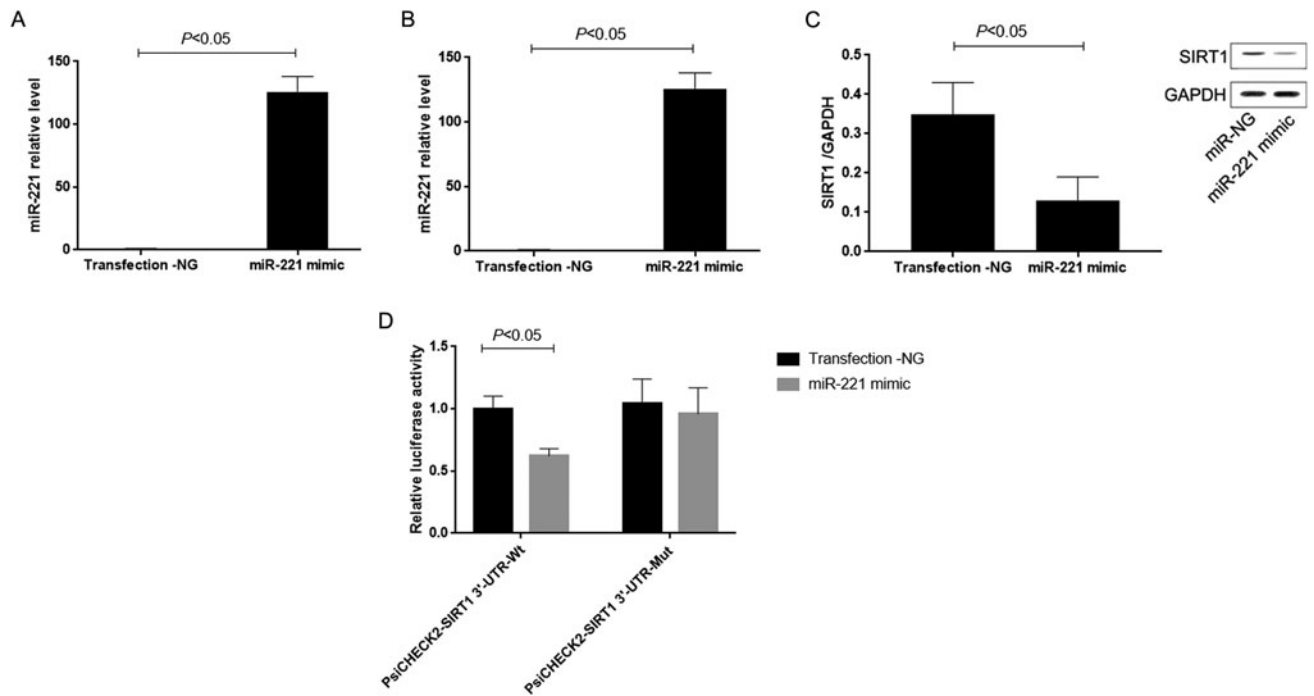


Figure 2. Overexpression of miR-221 could targetly inhibit SIRT1 expression in BEAS2B cells. miR-221 was up-regulated using mimics (A). SIRT1 mRNA and protein expression were inhibited after miR-221 up-regulation (B and C). Dual-luciferase reporter assay indicated that miR-221 could bind to the 3'-UTR of SIRT1. Data represent Mean $\pm$ SD, $n = 3$.

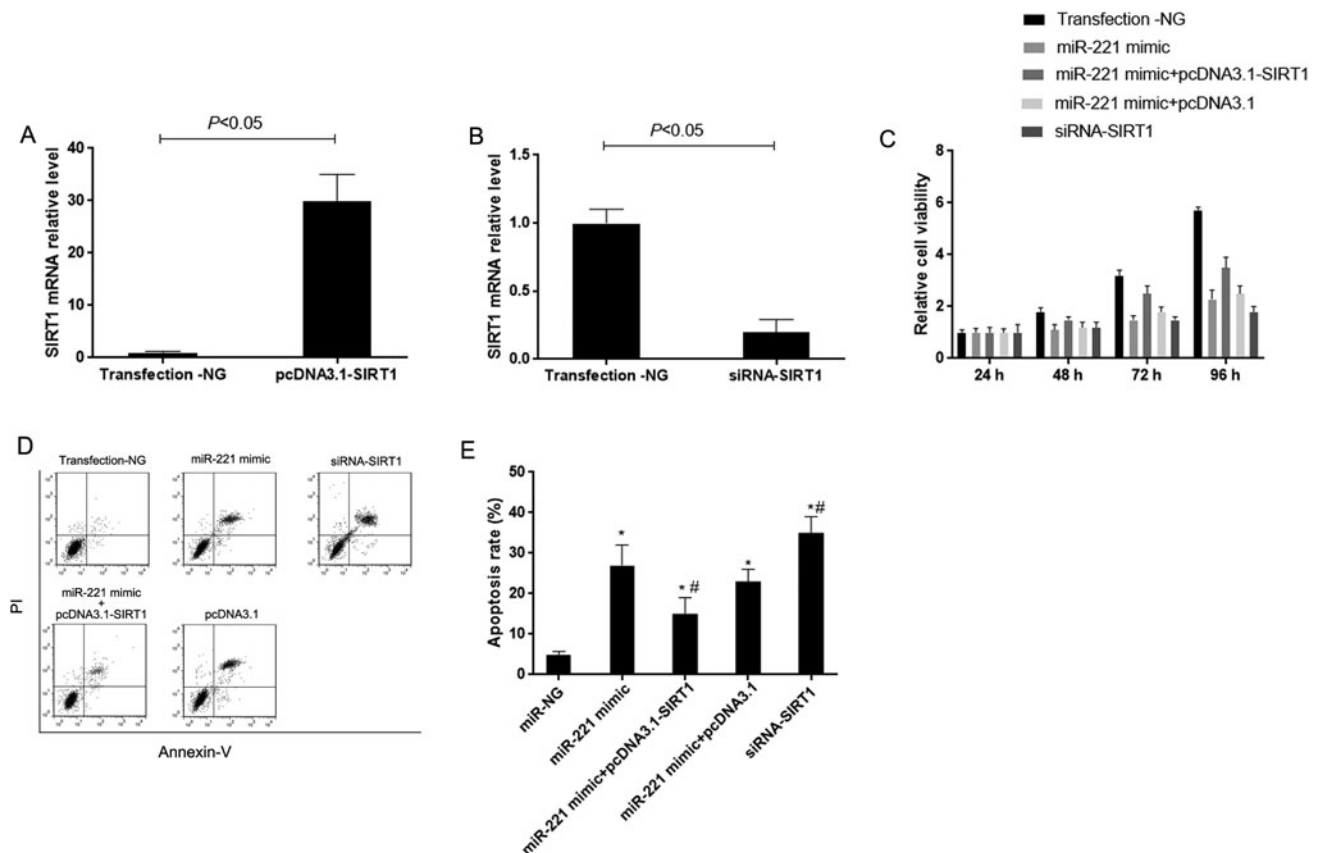


Figure 3. miR-221 inhibits the proliferation and induce apoptosis of BEAS2B cells by targeting SIRT1. pcDNA3.1-SIRT1 and siRNA-SIRT could up-regulate and knockdown of SIRT1 mRNA level in BEAS2B cells, respectively (B). Both SIRT1 knockdown and up-regulation of miR-221 decreased the cell viability of BEAS2B cells and induced apoptosis (C, D and E). Moreover, recovering SIRT1 level using pcDNA3.1-SIRT1 could rescue the cells treated by miR-221 mimic. * $P < 0.05$ compared with transfection negative control, # $P < 0.05$ compared with miR-221 mimic transfected cells. Data represent Mean $\pm$ SD, $n = 3$.

Discussion

Dysregulated expression of microRNAs have been well documented in asthma and a large amount microRNAs are proved that be associated with asthma's pathogenesis and clinical phenotypes such as airway inflammation, airway remodeling and airway hyper-responsiveness with the complicated mechanism.^{15,16} In brief, numerous studies have demonstrated that microRNA impact immune responses in asthma. Feng et al. reported miR-181a, miR-146a and miR-146b played proinflammatory roles in an ovalbumin (OVA)-induced murine model of asthma by moderate the CD4⁺ T lymphocytes.¹⁷ In our previous study and we found miR-145 could regulate the balance of Th1/Th2 through targeting the RUNX3 in asthma patients.¹⁸ Increasing evidence shows that microRNAs also have a vital effect on cells which compose airways such as airway smooth muscle cells and airway epithelial cells. For instance, Liu et al. addressed that elevating miR-221 significantly promote the proliferation and migration of human airway smooth muscle cell in asthma.¹⁹ Altered microRNA expression profile in airway epithelial cells during asthma was reported by Solberg et al. They supported that dramatic changes of microRNAs are a common feature of airway epithelial cells from a patient with asthma.²⁰ The level changes of miR-221 have been investigated by the previous study, much of these studies showed an up-regulated miR-221 in patients with asthma and regarded it as a disadvantageous factor for patients.²¹ miR-221 blockade suppressed airway inflammation in the OVA-induced murine asthma model.¹¹ Another study showed that miR-221 induced the hyper-proliferation of airway smooth muscle cells and regulated the release of IL-6 from patients with severe asthma. It indicated that blocking the expression of miR-221 may exert beneficial effects in severe asthma.²² However, whether miR-221 participates in the pathogenesis of airway epithelial cells in human asthma is still unclear. We undertook the present study to investigate the relevant role of miR-221. In the bronchoscopic brushing samples from asthmatic patients, we observed a higher level of miR-221, compared with healthy control. This result was consistent

with previous study and our hypothesis. In the *vitro*, we found overexpression of miR-221 could inhibit the proliferation of bronchial epithelial cells while promoting the apoptosis. It indicated that miR-221 may induce airway epithelial cells injury. In eukaryotic cells, most of microRNAs have effect by induce mRNA degradation and/or inhibit mRNA translation, hence we consider that miR-221 has an above-mentioned effect via targeting certain mRNAs. We conducted a bioinformatics prediction and obtained a list of candidate mRNAs. Among them, we selected SIRT1 as an interesting gene.

SIRT1 is putative cytoprotection molecules that have been verified to be significantly dysregulated in a variety of epithelial cells injury induced by inflammatory responses or other pathological stress conditions like oxidative stress.²³ Although a consensus has been reached in the protective effect of SIRT1, the expression or activity changes of SIRT1 in injured cells is still a controversial issue. Some of the evidence showed that damage factors could reduce the expression of SIRT1 in epithelial cells and insufficient SIRT1 may promote cell death. For example, Baker et al. stated a reduction of SIRT1 in bronchial epithelial cells (BEAS2B cells) exposure to H₂O₂.²⁴ Wang et al. indicated that high glucose stress could induce down-regulation of SIRT1 in corneal epithelial cells and overexpressions of SIRT1 promote the injury repairing ability of cells.²⁵ In contrast, some other studies indicated that in order to maintain cells survival or inhibit apoptotic cells death, the SIRT1 expression or activity could compensatorily increase in cells under injury condition. For instance, Yao et al. reported that SIRT1 level significantly increased in H₂O₂ treated human lens epithelial cells and attenuated oxidative stress-induced apoptosis.²⁶ on the other hand, some researchers suggest that the expression or activity changes of SIRT1 are dynamic and depend on the intensity and duration of the damage factors. Ding's group found that in mouse type II alveolar epithelial cells SIRT1 expression was significantly elevated and peaked at 12h after paraquat treatment but gradually decreased.²⁷ In agreement with the view that airways epithelial cells also suffer from the injury induced by inflammatory responses and oxidative

stress in asthma, we considered the expression changes in airways epithelial cells collected from patients with asthma. In the cells from bronchoscopic brushing samples, we detected a decreased SIRT1 mRNA level in patients group. As mentioned above, we suspect that SIRT1 was a target gene of miR-221, hence this reduction of SIRT1 was our expected result. Moreover, this result indicated that SIRT1 may play a role in the airways epithelial cells injury in the pathologic process of asthma. To confirm the target relationship between SIRT1 and miR-221 we up-regulated the miR-221 level in BEAS2B cells *in vitro*. We found that SIRT1 mRNA and protein level were both decreased by miR-221 overexpression. Moreover, dual-luciferase reporter assay showed that miR-221 could directly target on the 3'UTR SIRT1 mRNA. These data indicated that in BEAS2B cells miR-226 could target on SIRT1. Knock-down SIRT1 inhibited proliferation and resulted in apoptosis. Most importantly, in the rescue experiments, we induce SIRT1 overexpression by pcDNA3.1-SIRT1 vectors in miR-226 mimic transfected cells, compared with only miR-221 mimic transfection, cells transfected with miR-221 mimic + pcDNA3.1-SIRT1 has higher cell viability but lower apoptosis. It suggests that up-regulation of SIRT1 could attenuate the inhibitory effect of miR-221 on BEAS2B cells.

In summary, evidence from the present study supported that the miR-221 participate the airway epithelial cells injury in asthma via targeting SIRT1. The limitation of the present study is we did not explain why miR-221 increased in asthma. Currently, available therapies for allergic asthma are usually effective, but not enough for treating severe asthma. microRNA-based therapeutics may be a useful strategy for improving the curative effect of severe asthma. Considering the essential role of airway epithelial cells in asthma pathogenesis, we believe miR-221 may be a potential therapeutic target in asthma.

Acknowledgments

The study was supported solely by departmental funding.

Declaration of interest

The authors report no conflicts of interest.

References

1. Szymczak I, Wieczfinska J, Pawliczak R. Molecular background of miRNA role in asthma and COPD: An updated insight. *Biomed Res Int*. 2016;2016:7802521.
2. Gon Y, Hashimoto S. Role of airway epithelial barrier dysfunction in pathogenesis of asthma. *Allergol Int*. 2018;67(1):12–17.
3. Mertens TCJ, Karmouty-Quintana H, Taube C, Hiemstra PS. Use of airway epithelial cell culture to unravel the pathogenesis and study treatment in obstructive airway diseases. *Pulm Pharmacol Ther*. 2017;45:101–113.
4. Mitchell PD, O'Byrne PM. Epithelial-derived cytokines in asthma. *Chest*. 2017;151(6):1338–1344.
5. Khan MA, Assiri AM, Broering DC. Complement mediators: key regulators of airway tissue remodeling in asthma. *J Transl Med*. 2015;13:272.
6. Singhera GK, MacRedmond R, Dorscheid DR. Interleukin-9 and -13 inhibit spontaneous and corticosteroid induced apoptosis of normal airway epithelial cells. *Exp Lung Res*. 2008;34(9):579–598.
7. Rupani H, Sanchez-Elsner T, Howarth P. MicroRNAs and respiratory diseases. *Eur Respir J*. 2013;41(3):695–705.
8. Liu Z, Zhang XH, Callejas-Diaz B, Mullol J. MicroRNA in United Airway diseases. *Int J Mol Sci*. 2016;17(5):716.
9. Svitich OA, Sobolev VV, Gankovskaya LV, Zhigalkina PV, Zverev VV. The role of regulatory RNAs (miRNAs) in asthma. *Allergol Immunopathol (Madr)*. 2018;46(2):201–205.
10. Liu F, Qin HB, Xu B, Zhou H, Zhao DY. Profiling of miRNAs in pediatric asthma: upregulation of miRNA-221 and miRNA-485-3p. *Mol Med Rep*. 2012;6(5):1178–1182.
11. Qin HB, Xu B, Mei JJ, et al. Inhibition of miRNA-221 suppresses the airway inflammation in asthma. *Inflammation*. 2012;35(4):1595–1599.
12. Cetrullo S, D'Adamo S, Tantini B, Borzi RM, Flamigni F. mTOR, AMPK, and Sirt1: Key players in metabolic stress management. *Crit Rev Eukaryot Gene Expr*. 2015;25(1):59–75.
13. Beane J, Cheng L, Soldi R, et al. SIRT1 pathway dysregulation in the smoke-exposed airway epithelium and lung tumor tissue. *Cancer Res*. 2012;72(22):5702–5711.
14. Wang Y, Li D, Ma G, et al. Increases in peripheral SIRT1: A new biological characteristic of asthma. *Respirology*. 2015;20(7):1066–1072.
15. Foster PS, Plank M, Collison A, et al. The emerging role of microRNAs in regulating immune and

- inflammatory responses in the lung. *Immunol Rev.* 2013;253(1):198–215.
16. Greene CM, Gaughan KP. microRNAs in asthma: potential therapeutic targets. *Curr Opin Pulm Med.* 2013;19(1):66–72.
 17. Feng MJ, Shi F, Qiu C, et al. MicroRNA-181a, -146a and -146b in spleen CD4⁺ T lymphocytes play proinflammatory roles in a murine model of asthma. *Int Immunopharmacol.* 2012;13(3):347–353.
 18. Fan L, Wang X, Chen Q, et al. MicroRNA-145 influences the balance of Th1/Th2 via regulating RUNX3 in asthma patients. *Exp Lung Res.* 2016;42(8–10): 417–424.
 19. Liu G, Friggeri A, Yang Y, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med.* 2010;207(8):1589–1597.
 20. Solberg OD, Ostrin EJ, Love MI, et al. Airway epithelial miRNA expression is altered in asthma. *Am J Respir Crit Care Med.* 2012;186(10):965–974.
 21. Pua HH, Ansel KM. MicroRNA regulation of allergic inflammation and asthma. *Curr Opin Immunol.* 2015; 36:101–108.
 22. Perry MM, Baker JE, Gibeon DS, Adcock IM, Chung KF. Airway smooth muscle hyperproliferation is regulated by microRNA-221 in severe asthma. *Am J Respir Cell Mol Biol.* 2014; 50(1):7–17.
 23. Yang L, Duan Z, Liu X, Yuan Y. N-acetyl-l-cysteine ameliorates the PM2.5-induced oxidative stress by regulating SIRT-1 in rats. *Environ Toxicol Pharmacol.* 2018;57:70–75.
 24. Baker JR, Vuppusetty C, Colley T, et al. Oxidative stress dependent microRNA-34a activation via PI3K α reduces the expression of sirtuin-1 and sirtuin-6 in epithelial cells. *Sci Rep.* 2016;6:35871.
 25. Wang Y, Zhao X, Shi D, et al. Overexpression of SIRT1 promotes high glucose-attenuated corneal epithelial wound healing via p53 regulation of the IGFBP3/IGF-1R/AKT pathway. *Invest Ophthalmol Vis Sci.* 2013;54(5):3806–3814.
 26. Zheng T, Lu Y. SIRT1 protects human lens epithelial cells against oxidative stress by inhibiting p53-Dependent Apoptosis. *Curr Eye Res.* 2016;41(8): 1068–1075.
 27. Ding YW, Zhao GJ, Li XL, et al. SIRT1 exerts protective effects against paraquat-induced injury in mouse type II alveolar epithelial cells by deacetylating NRF2 in vitro. *Int J Mol Med.* 2016;37(4):1049–1058.