

Research Paper

ITGB4 deficiency induces mucus hypersecretion by upregulating MUC5AC in RSV-infected airway epithelial cells

Xizi Du^{1#}, Yu Yang^{1#}, Ming Yang², Lin Yuan¹, Leyuan Wang¹, Mengping Wu¹, Kai Zhou¹, Wenkai Li³, Yang Xiang¹, Xiangping Qu¹, Huijun Liu¹, Xiaoqun Qin¹, Chi Liu^{1,4}✉

1. Department of Physiology, School of Basic Medicine Science, Central South University, Changsha, Hunan, China
2. Centre for Asthma and Respiratory Disease, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle and Hunter Medical Research Institute, Callaghan, New South Wales, Australia
3. Department of Pediatrics, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha, Hunan, China
4. Research Center of China-Africa Infectious Diseases, Xiangya School of Medicine Central South University, Changsha, Hunan, China

#Contributed equally.

✉ Corresponding author: Chi Liu, Department of Physiology, School of Basic Medicine Science, Central South University at Changsha, Hunan, China. 410078. Tel. 86-73182355051, Fax. 86-73182355056, E-mail: liu.chi@csu.edu.cn.

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Received: 2021.08.18; Accepted: 2021.10.20; Published: 2022.01.01

Abstract

Respiratory syncytial virus (RSV) infection is the main cause of bronchiolitis in children. Excessive mucus secretion is one of the primary symbols in RSV related lower respiratory tract infections (RSV-related LRTI), which is closely associated with the occurrence and development of asthma in later life. Integrin $\beta 4$ (ITGB4) is down-regulated in the airway epithelial cells (AECs) of asthma patients which plays a critical role in the pathogenesis of asthma. However, whether ITGB4 is involved in the pathological processes of RSV infection remains unclear. In this study, we found that decreased expression of ITGB4 was negatively correlated with the level of MUC5AC in childhood AECs following RSV infection. Moreover, ITGB4 deficiency led to mucus hypersecretion and MUC5AC overexpression in the small airway of RSV-infected mice. MUC5AC expression was upregulated by ITGB4 in HBE cells through EGFR, ERK and c-Jun pathways. EGFR inhibitors treatment inhibited mucus hypersecretion and MUC5AC overexpression in ITGB4-deficient mice after RSV infection. Together, these results demonstrated that epithelial ITGB4 deficiency induces mucus hypersecretion by upregulating the expression of MUC5AC through EGFR/ERK/c-Jun pathway, which further associated with RSV-related LRTI.

Key words: respiratory syncytial virus, MUC5AC, ITGB4, airway epithelial cell

Introduction

Respiratory syncytial virus (RSV) is one of the most common respiratory pathogens causing lower respiratory tract infection among neonates and infants. RSV infection results about 33 million acute lower respiratory tract infections (LRTI) cases worldwide each year. Not only that, RSV-related LRTI would induce subsequent severe respiratory diseases and around 55,000–199,000 deaths in children younger than the age of 5 years old, which poses a heavy world medicine burden [1].

Airway epithelial cells (AECs), specifically ciliated AECs, is the main target for RSV infection. Of

particular concern is that mucus hypersecretion in AECs is one of most important pathological events in severe LRTI, which is always associated with increased risk of subsequently wheezing and later asthma in childhood [2, 3]. Mucus is important for the mucociliary clearance system, which is the first line against exogenous stimuli and pathogens [4]. However, mucus hypersecretion in pathological state would aggravate inflammatory response, which further led to airway hyperresponsiveness (AHR), airflow obstruction and weakened lung function [5]. Thus, exploring the intrinsic mechanism that causes

abnormal mucus secretion in RSV-infected AECs will contribute to prevent and reduce the clinically occurrence of severe LRTI.

Mucins, the main components of mucus, have many different types. In addition to forming a protective barrier against pathogens, mucins also have other unconventional functions, such as anti-inflammatory action and receptors for bacterium [6]. MUC5AC and MUC5B are the principal secretory mucins in the airway and MUC5AC is the primary gene expressed by airway epithelial goblet cells. Generally, MUC5AC is mainly expressed at relatively low level. However, the expression of MUC5AC increased significantly in inflammatory microenvironment which was usually caused by various external stimuli including RSV infection [6, 7]. Studies have found that MUC5AC overexpression induced the hypersecretion of airway mucus in RSV-infected AECs [8, 9]. However, the mechanisms underlying MUC5AC overexpression in RSV-infected AECs remain largely unclear.

Integrin family is a type of adhesion molecules that are constitutively expressed in AECs. Among these molecules, integrin $\beta 4$ (ITGB4) deserves a special attention for its unique intracellular long subunit. ITGB4 has been proven to act a critical role in the activation of phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinases (ERK1/2), nuclear factor kappa-B (NF- κ B) and other intracellular signaling pathways [10-12]. Of note, expression of ITGB4 decreased significantly by specific variation sites in the AECs of asthma patients [13]. Moreover, ITGB4 deficiency induces exaggerated lung inflammation and enhanced AHR in House Dust Mite (HDM) treated mice [14].

Although the association between ITGB4 and asthma has been verified, little is known about the contribution of ITGB4 to mucus hypersecretion after RSV infection in childhood asthma. Thus, we further thought to unravel the role of ITGB4 in the regulation of mucus production following RSV infection in AECs. First, RSV-infected model was constructed both *in vitro* and *in vivo* to investigate mucus production in ITGB4 deficient AECs following RSV infection. Then, throat swab samples from children's cases of RSV induced bronchiolitis and control cases were collected to verify the correlation between ITGB4 and MUC5AC in RSV-infected airway epithelia. Finally, corresponding intervention strategies were performed to identify the underpinning molecular mechanisms of MUC5AC overexpression in ITGB4 deficient AECs after RSV infection.

Material and methods

Throat swab sample collection

All participants provided written informed consent, approved by the No.2020KT-52 Central South University Research Ethics Committee. Children cases of RSV infection and healthy controls were recruited at Hunan Provincial People's Hospital between September 2020 and February 2021. Eligible patients (RSV patients' group) were aged under 18 and diagnosed with bronchiolitis and RSV-RNA positive. Patients who had pneumonia, acute heart failure within a month were excluded. Healthy controls were aged under 18 without respiratory disease. The demographic and clinical characteristics are shown in Table S2. Throat swab samples were collected for extracting RNAs and qPCR analysis by using BIOG RNA Swab Kit (BAIDAI, China).

Animals and challenge protocols

This animal studies were approved by the Xiangya Animal Protection and Use Committee of Central South University (No. 2020sydw0305). ITGB4 conditionally knocked out mice (ITGB4^{-/-} mice) was constructed according to our previous work [15]. The AECs-specific ITGB4 knock out was controlled by doxycycline (Dox) induction. After birth, Dox was placed in drinking water (1mg/ml) for mother. Infant mice are breastfed and injected with Dox (i.p. 25mg/kg) on day 7 and 14 after birth. ITGB4^{fl/fl} male littermates lacking either CCSP-rtTA or TetO-Cre were served as controls (ITGB4^{+/+} mice) which were treated with Dox synchronously. For the induction of RSV infection, mice were intranasally (i.n) stimulated with 1×10^6 pfu RSV-A2 in 20 μ l on day 15 after birth, and the control group received an equal volume of PBS [16]. Lung tissues were collected on day 17, 19, and 21 after birth (mice killed by exsanguination following an overdose of sodium pentobarbital). All the animal work was taken place in Department of Laboratory Animals, Central South University.

PAS staining

The PAS staining was performed following Glycogen Periodic Acid Schiff (PAS/Hematoxylin) Stain Kit (Solarbio, China) protocol [17].

Cell culture, transfection of siRNA, RSV infection, inhibitors treatment

Human bronchial epithelial (HBE) cells were purchased from Lifeline Cell Technology (Frederick, MD, USA), HBE cells were cultivated in DMEM with 10%FBS, 50U/ml Penicillin, 50U/ml Streptomycin. ITGB4 siRNA (50nM) or negative control siRNA (NC siRNA, 50nM) (RiboBio Inc, Guangzhou, China) were

transfected into HBE cells with LipofectAMINE 3000 (Invitrogen, Carlsbad, CA, USA). For the induction of RSV stimulation, HBE cells were incubated with RSV-A2 for 24 hours. In the group of inhibitor treatment, cells were pretreated for 30 min with AG1478 (1 μ M, EGFR phosphorylation inhibitor), U0126 (10 μ M, ERK phosphorylation inhibitor) or SP600125 (5 μ M, JNK inhibitor) (Selleck Chemicals, USA) respectively before incubation with RSV-A2.

RNA extraction, RT-PCR and qPCR

Total RNA was extracted by Trizol and quantified on a Varioskan microplate reader (Thermo Scientific, USA). PCR and qPCR were conducted according to the Kit protocol (PrimeScript RT Master Mix Kit, Takara, Japan) [15]. Primer sequences were shown in Table S1.

Western blot analysis

Protein was extracted from HBE cells according to previous procedures [18]. The following antibodies were used to determine the expression of corresponding protein: ITGB4 (ab197772, Abcam, USA), EGFR (ab52894, Abcam, USA), p-EGFR (ab32430, Abcam, USA), ERK1/2(ab184699, Abcam, USA), p-ERK1/2(ab223500, Abcam, USA), c-Jun (sc-74543, Santa Cruz, USA), p-c-Jun (sc-822, Santa Cruz, USA). Lamin- β 1(ab133741, Abcam, USA) and β -actin (ab8226, Abcam, USA) were used as corresponding controls, respectively.

Immunoprecipitation

Immunoprecipitation was performed according to our previous publications [19]. Briefly, protein from HBE cells was extracted with weak RIPA lysis buffer. An appropriate dilution of anti-ITGB4 antibody (Ab197772, Abcam, USA) or anti-EGFR antibody (sc-373746, Santa Cruz, USA) were added to the centrifuge tube which conjugate with suspension of Protein G. Mix the antibody-bead mixture at 4°C for 4 h using tube rotator. Then, 50 μ g of cell lysates were added into the mixtures and the lysate-bead/antibody conjugate mixtures were incubated overnight at 4°C. Mixtures were further resuspended in 5xSDS loading buffer. Samples were boiled for 5 minutes and analyzed by Western blot. Immunocomplexes were stained with anti-ITGB4 (ab197772, Abcam, USA) as well as EGFR (sc-373746, Santa Cruz, USA).

Immunocytochemical staining

HBE cells slides were fixed with 4% paraformaldehyde and incubated with PBS (containing 0.1% Triton X-100) for 10 minutes. After blocking with 1% BSA, cells were incubated with antibodies for MUC5AC (sc-21701, Santa Cruz, USA)

or c-Jun (sc-74543, Santa Cruz) in a humidified box and incubate overnight at 4°C. Then, cells were incubated with DAPI for DNA staining (Solarbio, China). Results were visualized using a fluorescence microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany).

Statistical analysis

Pearson' correlation was used to access the association between the expression of ITGB4 and MUC5AC, which was done with SPSS.19. Other data were analyzed with GraphPad Prism Software (Version 7; San Diego, CA) and presented as mean \pm SEM. Statistical comparisons were made by one-way ANOVA followed by Dunnett's post hoc test. Differences were considered statistically significant for *p < 0.05, **p < 0.01 and ***p < 0.001.

Results

ITGB4 deficiency in AECs induced mucus hypersecretion in RSV-infected mice

To explore the influence of ITGB4 deficiency on the mucus secretion in airway epithelia following RSV infection, RSV-infected mice animal was constructed (Figure 1A). PAS staining showed that, on day 2 post RSV infection, a small amount of mucus was detected in bronchial lumen of both ITGB4^{-/-} mice and ITGB4^{+/+} mice. On day 4 and day 6 post infection, goblet cells in the bronchial lumen of ITGB4^{-/-} mice were significantly proliferated and mucus secretion was greatly increased, whereas ITGB4^{+/+} mice showed no obvious goblet cell proliferation and mucus secretion (Figure 1B). Meanwhile, during days 4 to 6 post RSV infection, the expression of MUC5AC in ITGB4^{-/-} mice were significantly higher than that in ITGB4^{+/+} mice (Figure 1C, Figure S1).

Involvement of ITGB4 deficiency on the overexpression of MUC5AC in RSV patients

Both clinic and animal studies have shown that mucus hypersecretion from airway epithelia is one of most important pathological events in severe LRTI following RSV infection. To further determine the impact of ITGB4 on mucus hypersecretion, we examined the levels of MUC5AC through throat swab samples from RSV patients and healthy controls. Compared with healthy controls, expression of ITGB4 decreased significantly in RSV patients, (Figure 2A), while levels of MUC5AC were higher in RSV patients compared with healthy controls (Figure 2B). Of note, expression of ITGB4 was negatively associated with the level of MUC5AC in RSV patients (Figure 2C). This correlation strongly indicated that ITGB4 deficiency in airway epithelia was involved in mucus hypersecretion and MUC5AC overexpression

following RSV infection.

Level of MUC5AC was induced significantly in ITGB4 deficient HBE cells after RSV infection

To further verify the effect of mucus hypersecretion caused by ITGB4 deficiency, HBE cells were transfected with ITGB4 siRNA, respectively. The silence efficiency of ITGB4 was verified by western

blot (Figure 3A). After RSV infection, the mRNA expression of MUC5AC enhanced significantly in ITGB4 silenced HBE cells. Besides, comparing with control HBE cells, the protein expression of MUC5AC in ITGB4 silenced HBE cells also increased obviously following RSV infection (Figure 3B, C, D).

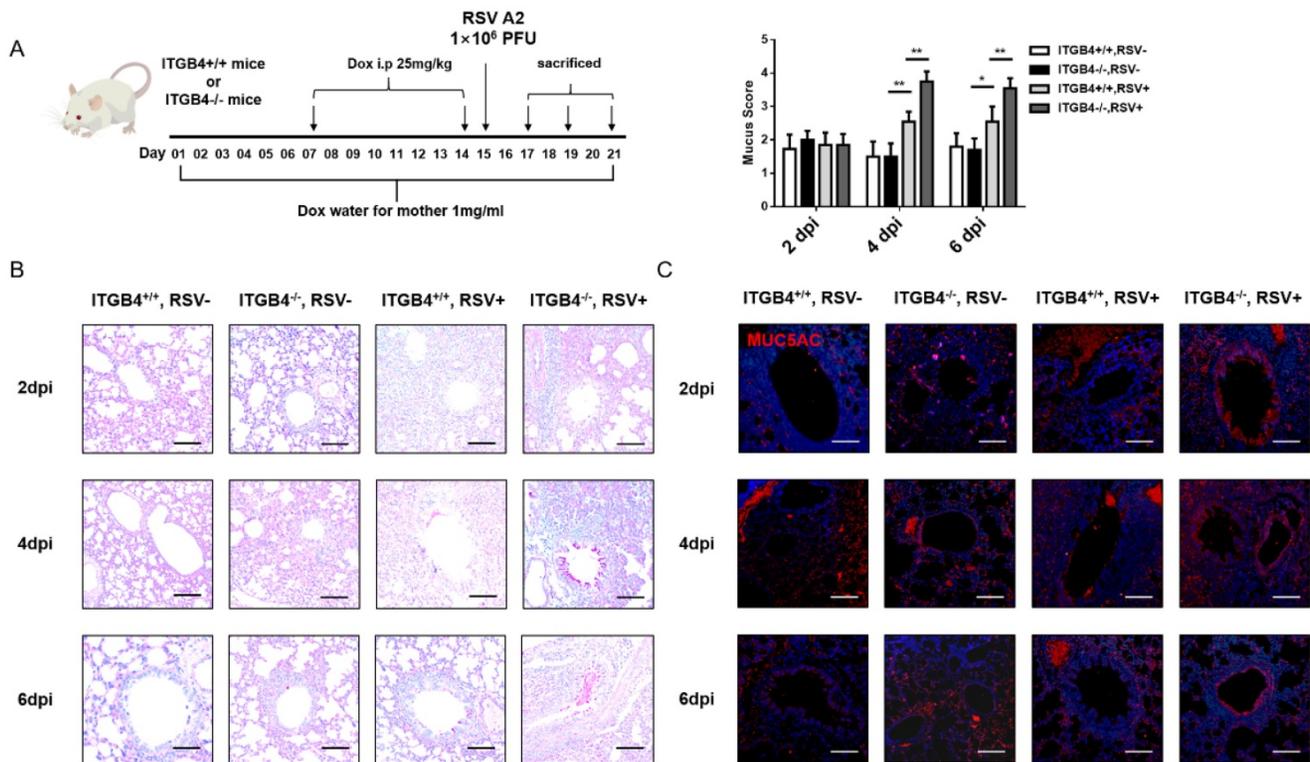


Figure 1. ITGB4 deficiency contributes to mucus hypersecretion and MUC5AC overexpression after RSV infection. A. Dox was placed in drinking water for mother and passed through breast milk to infant mice. Infant mice were injected Dox (i.p) on day 7 and 14 after birth. RSV was stimulated on day 15. Control mice received PBS. B. Mucus secretion were assessed by PAS staining. C. The level of MUC5AC were assessed by immunofluorescence (n = 10). Scale bar, 25 μ m. *p<0.1; **p<0.05.

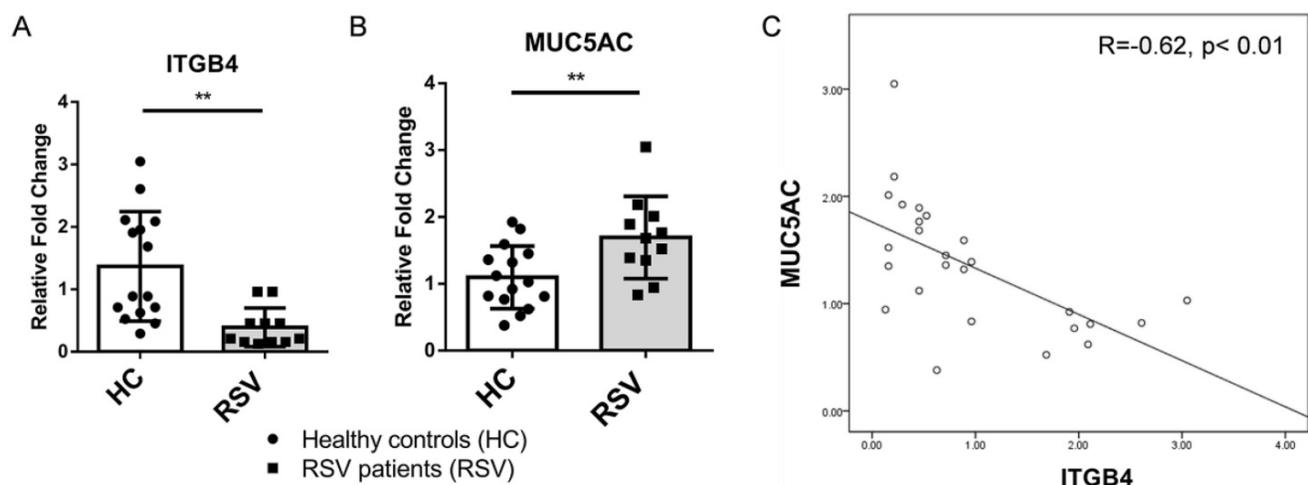


Figure 2. Expression of ITGB4 were negatively associated with level of MUC5AC in RSV patients. A. mRNA expression of ITGB4 in throat swab samples from RSV patients (n=11) and healthy controls (n=15). B. mRNA expression of MUC5AC in throat swab samples from RSV patients and healthy controls. C. Correlation analysis between ITGB4 expression and level of MUC5AC in RSV patients. **p<0.05.

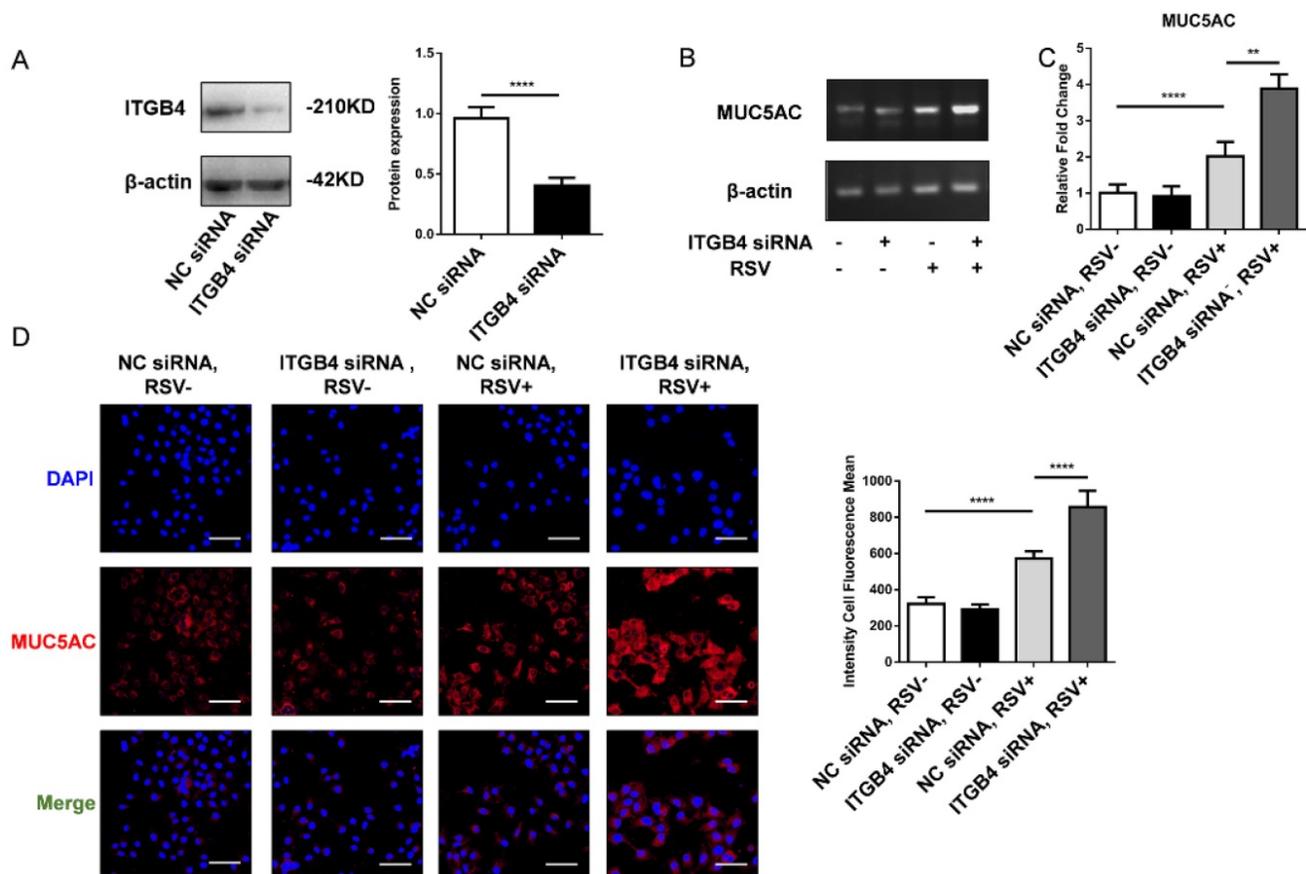


Figure 3. The level of MUC5AC increased significantly in ITGB4 silenced HBE cells after RSV infection. A. The silence efficiency of ITGB4 was verified by western blot. B-D. ITGB4 deficiency significantly upregulated the mRNA and protein level of MUC5AC, respectively. Immunofluorescence were quantified by the fluorescence intensity (n = 10). Scale bar, 50 μ m. Values are represented as mean \pm SEM of at least three independent experiments. ** p<0.05; **** p<0.001.

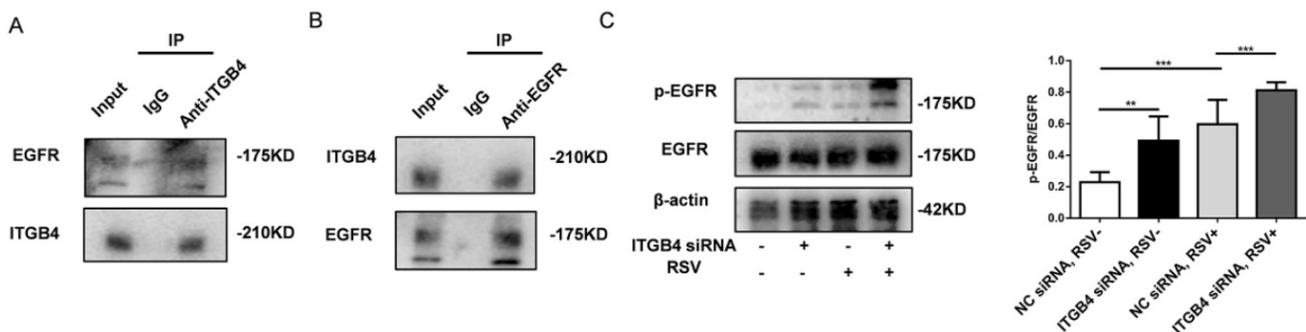


Figure 4. The interaction between ITGB4 and EGFR and the phosphorylation of EGFR in ITGB4 silenced HBE cells after RSV infection. A-B. Immunoprecipitation of RSV-infected HBE cells for interaction between ITGB4 and EGFR. C. ITGB4 deficiency enhanced EGFR phosphorylation in RSV-infected HBE cells, as compared with that in control HBE cells. Values are represented as mean \pm SEM of at least three independent experiments. ** p<0.05; *** p<0.01.

ITGB4 deficiency increased EGFR phosphorylation in RSV-infected HBE cells

Previous literatures have showed that ITGB4 reduces the binding of ligand to EGFR and the mobility of EGFR in the plasma membrane through physical interaction with EGFR [20-22]. In this study, we further determined whether ITGB4 regulates EGFR phosphorylation in RSV-infected HBE cells. Assay with immunoprecipitation revealed a direct interaction between ITGB4 and EGFR in RSV-infected

HBE cells (Fig 4A, B). Basal level of EGFR phosphorylation in RSV-infected HBE cells was higher than that in control HBE cells without outside stimuli. Moreover, after RSV infection, ITGB4 silence further enhanced EGFR phosphorylation in HBE cells compared with that in control HBE cells (Fig 4C). Either at baseline or after RSV infection, there is no significant difference of total EGFR between ITGB4 silenced HBE cells and control HBE cells. The above results indicated that ITGB4 was involved in the activation of EGFR during RSV infection.

ITGB4 deficiency enhanced the activation of EGFR/ERK/c-Jun pathway in RSV-infected HBE cells

To further explore the role of EGFR mediated signaling pathways in ITGB4 deficiency-induced MUC5AC overexpression, we examined the downstream pathway of EGFR activation in ITGB4 silenced HBE cells after RSV infection. The expression of ERK1/2, phosphorylated ERK1/2 (p-ERK1/2), c-Jun and phosphorylated c-Jun (p-c-Jun) were detected in ITGB4 silenced HBE cells after RSV infection using multiple inhibitors, including AG1478, U0126 and SP600125. Western blot analysis showed that treatment with AG1478, U0126 and SP600125 significantly inhibited the level of p-EGFR, p-ERK1/2 and p-c-Jun in ITGB4 silenced HBE cells after RSV infection, respectively (Figure 5A, B, C).

As c-Jun is an important binding site on MUC5AC promoter, we further verify the possible involvement of c-Jun in the overexpression of MUC5AC. The nuclear c-Jun subunit was detected upon stimulation with AG1478 or U0126. Immunofluorescence results demonstrated the

nuclear translocation of c-Jun in ITGB4 silenced HBE cells following RSV infection (Figure 6A). As expected, treatments with AG1478, U0126 reduced the nuclear translocation of c-Jun in ITGB4 silenced HBE cells after RSV infection (Figure 6B).

Blockade of EGFR/ERK1/2/c-Jun pathway inhibited MUC5AC overproduction in ITGB4 silenced HBE cells after RSV infection

To further verify the involvement of EGFR-ERK1/2-c-Jun pathway in the regulation of MUC5AC expression, we detected the level of MUC5AC in ITGB4 silenced HBE cells after RSV infection using AG1478, U0126, SP600125, respectively. Administration of AG1478, U0126 and SP600125 significantly blocked the phosphorylation of EGFR, ERK, c-Jun in ITGB4 silenced HBE cells (Figure 5A, B, C). More importantly, these inhibitor treatments blocked MUC5AC overexpression in ITGB4 silenced HBE cells after RSV infection (Figure 7A, B). These findings verified that ITGB4 deficiency upregulate MUC5AC expression through activation of EGFR/ERK/c-Jun pathway in RSV-infected HBE cells.

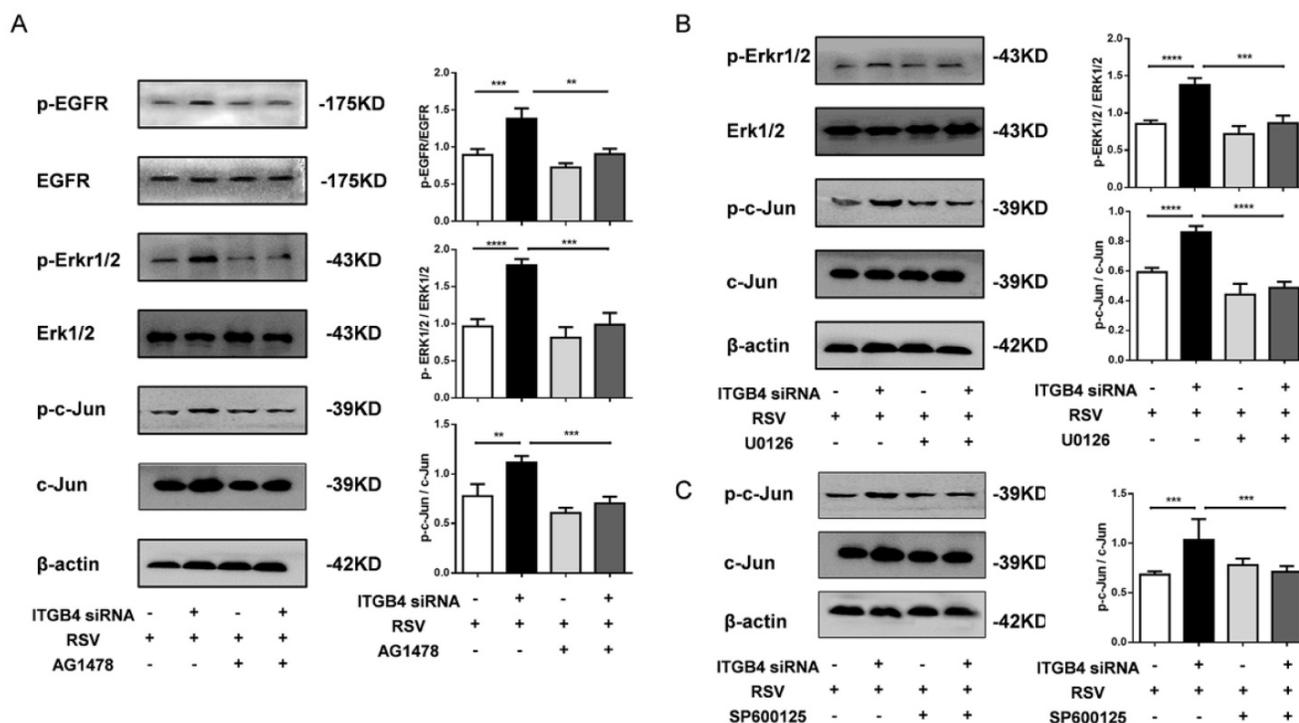


Figure 5. ITGB4 deficiency leads to augmented activation of EGFR/ERK1/2/c-Jun pathway. A. AECs from different group was pretreated with AG1478 and then infected with RSV. Western blot analysis of the expressions of p-EGFR, EGFR, p-ERK1/2, ERK1/2, p-c-Jun, c-Jun. B. HBE cells from different group was pretreated with U0126 and then infected with RSV. Western blot analysis of the expressions of p-ERK1/2, ERK1/2, p-c-Jun, c-Jun; C. HBE cells from different group was pretreated with SP600125 and then infected with RSV. Western blot analysis of the expressions of p-c-Jun and c-Jun; Values are represented as mean \pm SEM of at least three independent experiments. ** $p < 0.05$; *** $p < 0.01$; **** $p < 0.001$.

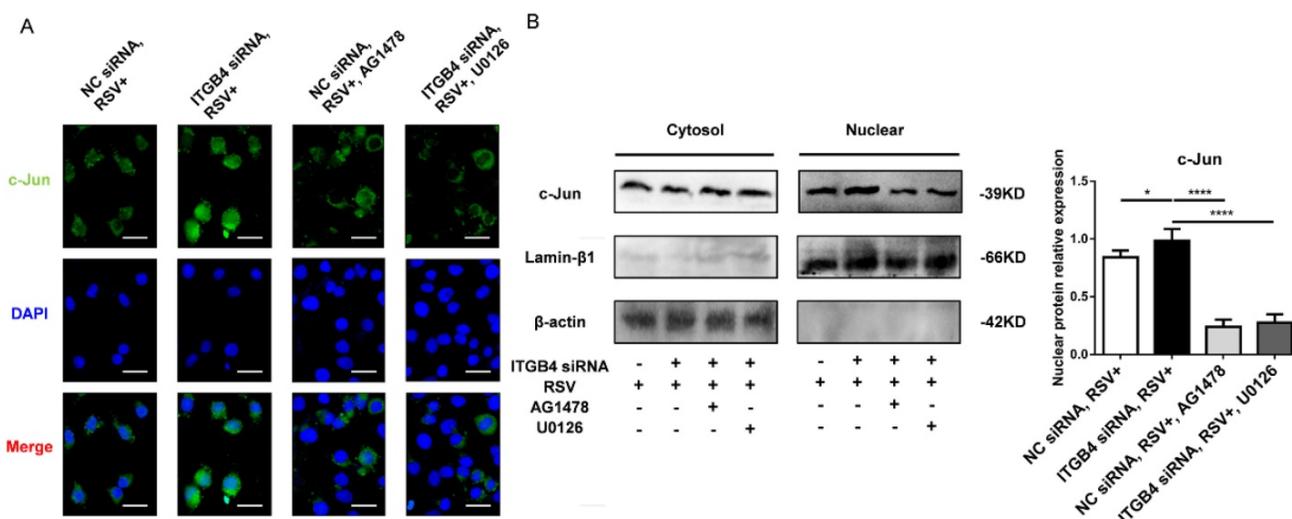


Figure 6. Nuclear extract for c-Jun was detected in ITGB4 silenced HBE cells after RSV infection under treatment with AG1478 and U0126, respectively. A. Immunofluorescence analyzed the localization and expressions of c-Jun (n = 10). Scale bar, 100 μ m. B. Western blot analysis of c-Jun.

Inhibition of EGFR phosphorylation reduced mucus hypersecretion and MUC5AC overexpression in ITGB4^{-/-} mice after RSV infection

As the key role for EGFR is indicated in the pathogenesis of mucus secretion, we block EGFR phosphorylation with AG1478 in ITGB4^{-/-} mice after RSV infection. On day 6 post RSV infection, treatment with AG1478 significantly decreased both mucus hypersecretion in airway lumen and MUC5AC overexpression in airway epithelia (Figure 8A, B). These results verified that EGFR signaling pathway underpins ITGB4-regulated MUC5AC expression in AECs which further contributes to mucus hypersecretion after RSV infection.

Discussion

Airway mucus is the first biological hydrogel barrier of airway to defend against outside insults. Mucin, as a critical component of mucus, plays an important role in innate immunity which goes beyond the scope of mucociliary. MUC5AC and MUC5B are the principal gel secretory mucins which are important hallmarks of airway pathological state. MUC5B mainly exerts a role in ensuring normal mucus clearance, whereas MUC5AC is the main up-regulated mucin which was involved in the development of AHR, mucous metaplasia, and airway mucus plugging. Moreover, overexpression of MUC5AC is also accompanied with the inducement of airway inflammation [7, 23, 24]. It is well known that the expression of MUC5AC in AECs increased obviously in allergic asthma patients [25]. Animal models further demonstrated that MUC5AC deficiency enhanced mucociliary transport and antibacterial defense in AECs [26]. Meanwhile, mucus

plugs were significantly reduced in MUC5AC-deficient mice after allergen stimulation [27]. In addition, MUC5AC overexpression in AECs could also imply airway goblet cell metaplasia, which is a key feature of airway remodeling in asthma [28, 29].

Notably, RSV infection is the major trigger for airway immune response and mucus production, which further led to airway obstruction, AHR and airway inflammation. A large body of evidence from epidemiologic studies have shown that RSV infection in infancy and early childhood was associated with elevated susceptibility of later asthma [30]. Early RSV infection may have sustained effects on subsequent local lung immune responses [31]. Moreover, RSV infection and asthma may share a common host genetic background [30, 32]. Intriguingly, there is an obvious heterogeneity in the severity of RSV infection, which may be related to the susceptibility of subsequent asthma. However, the intrinsic mechanism underlying the heterogeneity of RSV infection is far from unclear.

Our previous work found that ITGB4 deficiency in AECs was involved in the development of Th2 responses of allergic asthma though ITGB4-mediated signaling events [13, 33]. In this present study, we further pointed out that the decreased expression of ITGB4 was negatively correlated with the level of MUC5AC in throat swab of children with RSV infection. Moreover, ITGB4 deficiency enhances mucus hypersecretion and MUC5AC overexpression following RSV infection in mice model. Furthermore, our findings identify the underpinning molecular mechanisms of mucus hypersecretion in ITGB4 deficient AECs after RSV infection and highlight the potential of targeting this pathway for the treatment of mucus hypersecretion.

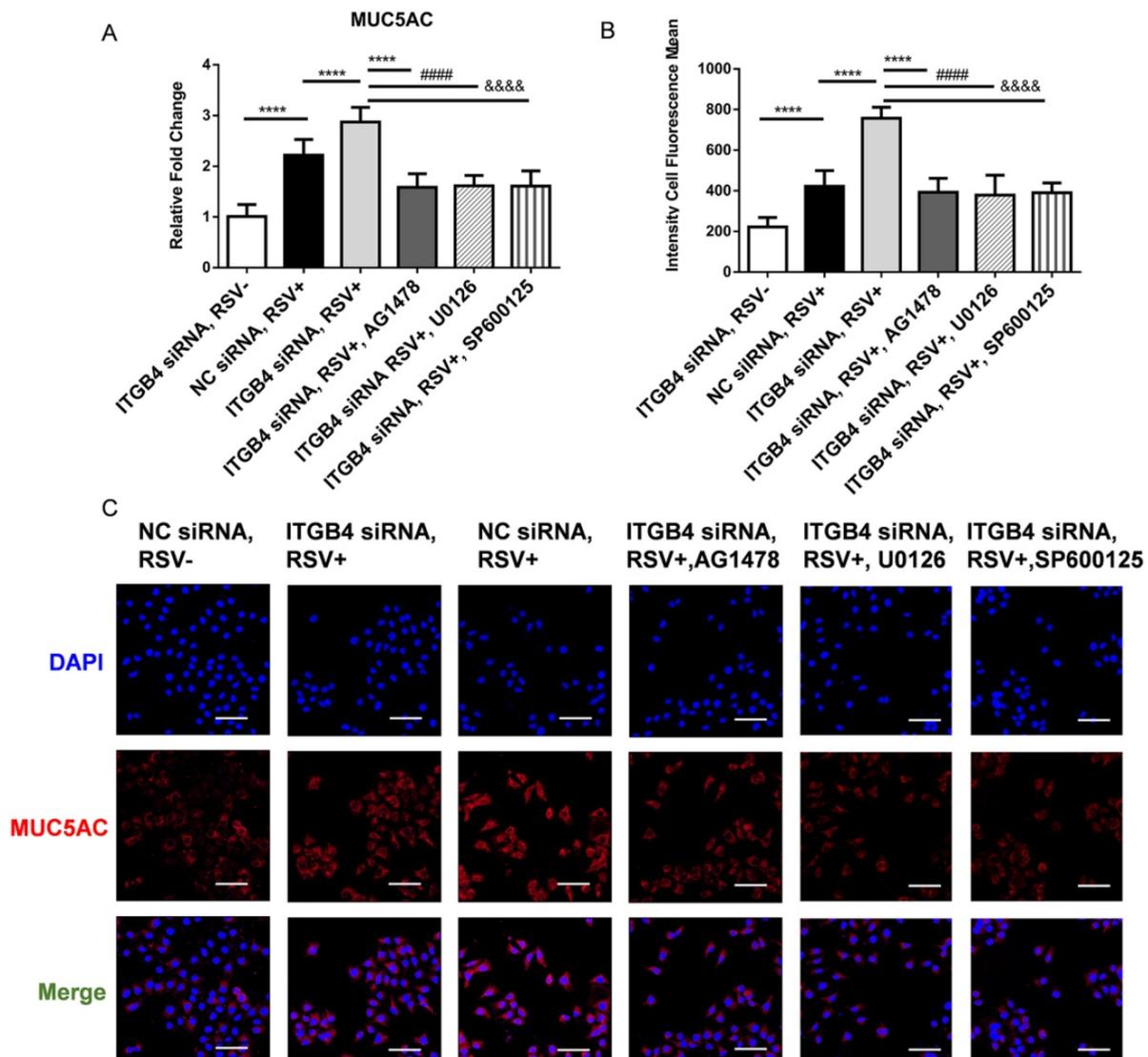


Figure 7. Block of EGFR phosphorylation, ERK1/2 phosphorylation and c-Jun phosphorylation inhibited MUC5AC overexpression in ITGB4 silenced HBE cells after RSV infection. A. qPCR analysis of MUC5AC mRNA expression. B. Level of MUC5AC was assessed by immunofluorescence and quantified by the fluorescence intensity ($n = 10$). Scale bar, 50 μm . Values are represented as mean \pm SEM of at least three independent experiments. **** $p < 0.001$.

Studies have reported that EGFR pathway is necessary for mucus production in AECs [34, 35]. The binding of EGF and EGFR in epithelial cells will be triggered after subjection to external stimuli. Activation of EGFR induced receptor dimerization and intracellular domain phosphorylation, which can further activate downstream regulators, including c-Jun N-terminal kinase (JNK), AKT, NF- κ B. Of note, the expression of MUC5AC is associated with the activation of c-Jun, AKT and NF- κ B pathway [36]. Besides, EGFR tyrosine kinase inhibition has also been shown to block the expression of MUC5AC. It has confirmed that EGFR phosphorylation was regulated by ITGB4 in gastric cancer, breast cancer and liver cancer [37]. In this study, we further demonstrated that ITGB4 deficiency in AECs induces overproduction of MUC5AC through EGFR activation, and

use of EGFR phosphorylation inhibitors could restore expression of MUC5AC significantly [20-22]. EGFR is a kind of receptor tyrosine kinase with intrinsic tyrosine kinase activity, which can induce phosphorylation of multiple intracellular substrates, of which ERK1/2 is the most widely known downstream pathway [38]. ERK1/2 is a central component in the MAPK decades. After activated by phosphorylated EGFR, ERK1/2 enters the nucleus and acts on transcription factors such as NF- κ B and AP-1 [39, 40]. AP-1, as a type of transcription factor, is composed by subunit c-Jun and subunit c-Fos, which is involved in basal transcription of MUC5AC [41]. Our results also proved that ITGB4 and EGFR have a direct interaction in human AECs, which is in line with previous findings [11, 22].

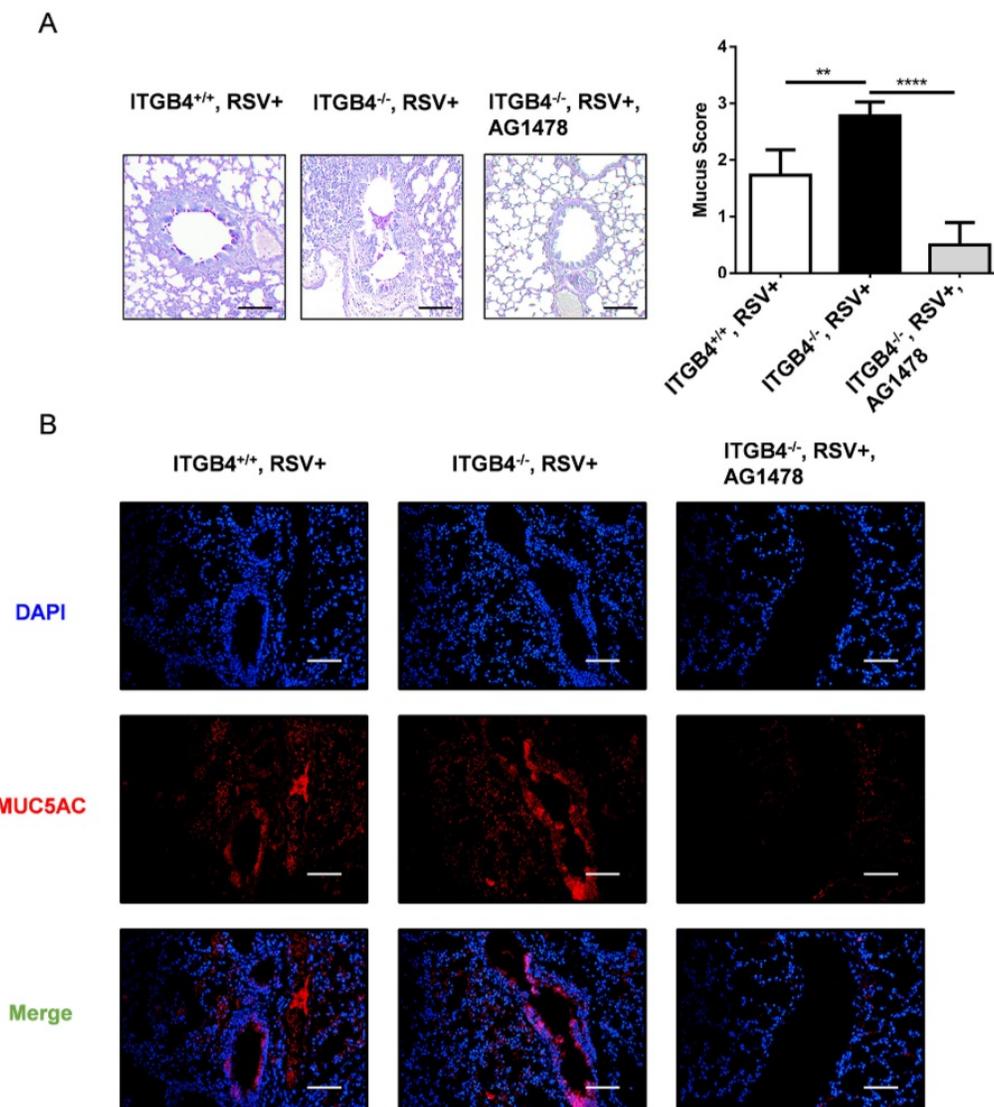


Figure 8. Inhibition of EGFR phosphorylation reduced mucus hypersecretion and MUC5AC overexpression in ITGB4^{-/-} mice after RSV infection. A. Mucus secretion were assessed by PAS staining. B. Level of MUC5AC were assessed by immunofluorescence (n = 10). Scale bar, 25 μ m. ** p<0.05; *** p<0.01; **** p<0.001.

Intriguingly, our data also found that ITGB4 deficiency in AECs increased the phosphorylation of EGFR slightly even without RSV infection. However, the level of MUC5AC was enhanced in ITGB4 deficient AECs only under RSV infection. This phenomenon suggests that the expression of MUC5AC was regulated mainly under specific stimulus, such as RSV. Moreover, apart from the ITGB4-EGFR pathways, other components pathways may also participate in the regulation of MUC5AC in AECs [42-44]. Not only that, Lakshmanan *et al.* have discovered that MUC5AC interacts directly with ITGB4 in genetically engineered mouse adenocarcinoma tissues [45]. However, such direct interaction between ITGB4 and MUC5AC was not observed in our primary AECs which may due to the distinct cellular functions and regulation mechanism of AECs.

In summary, this study found that decreased expression of ITGB4 in AECs was involved in mucus hypersecretion and MUC5AC overexpression through EGFR, ERK and c-Jun pathway following RSV infection. This could serve to better understand the mechanism of ITGB4 deficiency on mucus hypersecretion in RSV-infected AECs. Complete elucidation of the underlying mechanism might provide novel therapeutic targets to reduce mucus overproduction in RSV-related LRTI.

Abbreviations

RSV: Respiratory syncytial virus; AECs: airway epithelial cells; ITGB4: Integrin β 4; AHR: airway hyperresponsiveness; HDM: House Dust Mite.

Supplementary Material

Supplementary figure and tables.

<https://www.ijbs.com/v18p0349s1.pdf>

Acknowledgements

Funding Sources

This work was funded by grants #82070034, #81970033, #31900424 from the NSFC; grants #2021JJ30898, #2020JJ4776, #2020JJ4688, #2019JJ50760 from the Hunan Natural Science Foundation; grant #20K142 from open Foundation of Hunan College Innovation Program; and the grant for Open Sharing Fund for the Lager-scale Instruments and Equipment's of Central South University.

Availability of data and materials

All data used and analyzed in this study are included in this article.

Competing Interests

The authors have declared that no competing interest exists.

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