



Original article

KIF3A knockdown sensitizes bronchial epithelia to apoptosis and aggravates airway inflammation in asthma

Gang Geng^{a,b,c}, Ying Du^d, Jihong Dai^{a,b,c}, Daiyin Tian^{a,b,c}, Yunqiu Xia^{a,b,c}, Zhou Fu^{a,b,c,*}^a Department of Respiration, Children's Hospital of Chongqing Medical University, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing 400014, China^b China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing 400014, China^c Chongqing Key Laboratory of Pediatrics, Chongqing 400014, China^d Department of Gynecology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

ARTICLE INFO

Keywords:

KIF3A

Asthma

Bronchial inflammation

Epithelium

ABSTRACT

Background: KIF3A expression was decreased in asthmatic child patients and animal. Impaired KIF3A expression resulted in increased Th2 inflammation in mice and apoptosis in renal tubular epithelium and photoreceptor cells. This work aimed to investigate the role of KIF3A in epithelium apoptosis and bronchial inflammation in asthma.

Methods: After establishment of ovalbumin induced asthma, the mice were infected with KIF3A adenovirus through nasal cavity inhalation. KIF3A expression and apoptosis in epithelia of nasal mucosa and bronchia were determined using qRT-PCR, western blotting, immunohistochemistry and TUNEL staining. The mRNA expression of COX-2, IL-4, IL-5, IL-13, IL-6, IL-10 and TNF- α was also measured. *In vitro*, human bronchial epithelial cell line 16HBE 14o- was stimulated with IL-4, IL-13 and TNF- α , accompanied by KIF3A knockdown or overexpression using siRNA or KIF3A adenovirus respectively. Apoptosis, mRNA expression of CCL17, CCL26, IL-5 and IL-8, and protein expression of COX-2 and β -catenin were determined using flow cytometry, qRT-PCR and western blotting.

Results: KIF3A expression was reduced in epithelia of nasal mucosa and bronchia of asthmatic mice, and overexpression of KIF3A ameliorated epithelial cell apoptosis and bronchial inflammation in asthmatic mice. *In vitro*, KIF3A knockdown significantly promoted epithelium apoptosis, facilitated the transcription of CCL17, CCL26, IL-5 and IL-8, and increased the protein levels of COX-2 and β -catenin translocation, whereas overexpression of KIF3A exhibited the opposite effect.

Conclusion: KIF3A plays an important role in epithelium apoptosis and bronchial inflammation in asthma, and may be a potential target for asthma treatment.

1. Introduction

Asthma is one of the most common chronic immunological diseases in humans, affecting more than 300 million people worldwide, and at least 250,000 people die from this disease each year [1]. Airway type 2 immune responses is an important molecular mechanism of asthma, and is mainly mediated by eosinophils, mast cells, basophils, T helper 2 (Th2) cells, group 2 innate lymphoid cells (ILC2s) and IgE-producing B cells. After exposure to activation stimulus (such as oxidant, virus or allergen), airway epithelium release thymic stromal lymphopoietin (TSLP), IL-25 or IL-33, which result in increased production of type 2 cytokines (IL-4, IL-5 and IL-13). These cytokines drive a cascade of events including, IgE-triggered hypersensitivity to aeroallergens,

activation of airway epithelial cells, chemoattraction of effector cells (mast cells, eosinophils and basophils), and remodeling of the epithelium and subepithelial matrix [2]. Thus, inhibition of Th2 inflammation is an important strategy for asthma therapy. Glucocorticoids (GCs) are the mainstay controller medication for asthma due to its suppression effect on type 2 inflammation. However, GCs have multiple side effects, including dysphonia, candidiasis, cataracts, osteoporosis and adrenal suppression. Therefore, it is of great significance to screen type 2 inflammation related genes in asthma control.

The *kinesin family number 3A (KIF3A)* gene positioned on the human chromosomal region 5q31-33 region, a susceptibility locus for immune diseases including asthma [3–6], encodes a motor subunit of kinesin-2, an important component for the formation and mechanism of cilia. A

* Corresponding author at: Zhou Fu, Department of Respiration, Children's Hospital of Chongqing Medical University, No. 136 Zhongshan Two Road, Yuzhong District, Chongqing 400014, China.

E-mail address: a140122a@126.com (Z. Fu).

<https://doi.org/10.1016/j.bioph.2017.10.160>

Received 27 July 2017; Received in revised form 24 October 2017; Accepted 28 October 2017

0753-3322/ © 2017 Elsevier Masson SAS. All rights reserved.

recent research revealed that *KIF3A* deficient mice were highly susceptible to exposure to the aeroallergens *Aspergillus fumigatus* and House Dust Mite extracts, resulting in increased airway hyper-responsiveness and Th2-mediated, eosinophilic inflammation [7]. Combined with evidence that *KIF3A* mRNA was decreased in respiratory epithelial cells during clinical exacerbations in children with asthma, the author demonstrated *KIF3A* was required for type 2 inflammation suppression after aeroallergen exposure, and by which *KIF3A* regulates airway epithelial cell functions related to the pathogenesis of asthma in children [7]. A study by Melinda Kovacic et al. also found that *KIF3A* mRNA level was significantly downregulated in nasal epithelium from the asthmatic children and a house dust mite-induced murine asthma model, and they identified *KIF3A* as a novel candidate gene for childhood asthma using RNA expression and population allelic frequencies differences [8].

In addition to bronchial inflammation, aberrant apoptosis of airway epithelial cells (ECs) is also a disease contributing feature in the asthma. It has been shown that T cells and eosinophils contribute to the induction of ECs apoptosis by secretion of IFN- γ and TNF- α and triggering of death receptors on bronchial ECs [9]. *KIF3A* has also been involved in apoptosis. Studies showed that *KIF3A* knockdown led to a decrease in cilia and an increase in cisplatin induced apoptosis in renal tubular epithelial cells [10]. Loss of *KIF3A* also caused apoptotic cell death in photoreceptor cell [11]. However, whether the low expression of *KIF3A* in bronchial ECs also leads to its sensitivity to apoptosis is still unknown. Therefore, in this work, we attempted to investigate the role of *KIF3A* in ECs apoptosis under the stimulation of IL-4, IL-13 and TNF- α , and the effect of *KIF3A* overexpression on type 2 inflammation and airway epithelial structural integrity in asthmatic mice.

2. Materials and methods

2.1. Animals and treatments

This study was approved by the Ethics Committee of Children's Hospital of Chongqing Medical University. Thirty female BALB/C mice (5 weeks old), purchased from the Shanghai SLAC laboratory animal Co., Ltd. (Shanghai, China) were housed under conventional conditions for 1 week prior to any experiments. Then, the mice were randomly divided into three groups and receiving corresponding treatments. Normal group, the mice were sensitized and challenged by saline alone and without nasal infection of adenoviruses. Asthma model group (OVA), the mice were sensitized and challenged by ovalbumin (OVA) followed by nasal infection of empty vector adenoviruses. *KIF3A* overexpression group (OVA + *KIF3A* AdV), the mice were sensitized and challenged by OVA followed by nasal infection of vector adenoviruses carrying *KIF3A* gene.

2.2. Allergen immunization/challenge protocol

Mice received an *i.p.* injection of 200 μ l of sensitization fluid (containing 100 μ g of OVA and 20 mg of Alum) on day 0 and 14. From day 21 for 7 days, the mice were placed in a 20 cm * 30 cm * 50 cm semi closed transparent glass box and given 40 ml of OVA solution (2%) through pulverization inhalation for 30 min once a day to induce asthma. The mice in normal group were sensitized and challenged with 0.9% normal saline.

2.3. Infection of adenovirus

After being made asthmatic model by OVA-sensitization and challenge, the asthmatic mice in OVA + *KIF3A* AdV group were infected with 40 μ l of mouse *KIF3A* Adenovirus (1×10^7 PFU/ml) supplied by Vector BioLabs (ADV-262821) through nasal cavity inhalation under anesthesia. The mice in OVA group were infected with equivalent empty adenovirus (Vector BioLabs). The backbone is human adenovirus

type 5 (dE1/E3), and other specific construction information can be found in the official website (www.vectorbiolabs.com). Forty-eight hours after infection the animals were killed. Then the nasal and bronchi tissues were isolated as described previously for further analyses [12,13].

2.4. TUNEL staining

The nasal and bronchi tissues were rinsed with saline, fixed with 10% formalin, embedded in paraffin and cut at a thickness of 5 μ m. Apoptosis were detected by *in situ* labeling using a TUNEL kit (Sino-America, China) according to the manufacturer's instructions. The slides were evaluated by light microscopy and TUNEL-positive cells in five random visual fields were counted.

2.5. Immunohistochemical staining for *KIF3A*

For detection of *KIF3A*, paraffin sections were prepared as described above. After deparaffinization and rehydrated, the slides were blocked with 5% fetal bovine serum for 30 min at room temperature. Then, sections were incubated with primary antibody for *KIF3A* (dilution 1:100; Sigma, USA) overnight at 37 $^{\circ}$ C, and followed by incubation with biotinylated secondary anti-mouse antibody diluted 1:200 in 2% BSA for 30 min at room temperature, and detected with the ABC immunodetection kits (Vector Laboratories). Sections were counterstained with Harris hematoxylin solution (Sigma), passed through a dehydration process and cover slipped.

2.6. ELISA assay

The concentration of Th2 cytokines (IL-4, IL-5 and IL-13), pro-inflammatory cytokines (IL-6 and TNF- α), IL-8 and IL-10 in Broncho alveolar lavage fluid (BALF) were measured using Elisa kits according to the manufacturer's instructions. Elisa kits used were list as below: mouse IL-4 ELISA Kit (ab100710, Abcam), mouse IL-5 ELISA Kit (ab100711, Abcam), mouse IL-13 ELISA Kit (ab219634, Abcam), mouse IL-6 ELISA Kit (ab100712, Abcam), mouse TNF alpha ELISA Kit (ab100747, Abcam), mouse IL-8 ELISA Kit (H008, Nanjing Jiancheng Bioengineering Institute) and mouse IL-10 ELISA kit (ab108870, Abcam).

2.7. Cell culture and treatment

Human bronchial epithelial cell line 16HBE 14o- was purchased from American Type Culture Collection (ATCC) and maintained in Minimum Essential Medium (MEM) supplemented with 10% FCS in a humidified incubator at 37 $^{\circ}$ C with a 5% CO₂ atmosphere. After confluence, the cells were transferred into low serum medium (1% FCS) for 24 h for synchronization. The synchronized cells were subsequently transfected with small interfering RNA (siRNA) targeting *KIF3A* or adenovirus (AdV) carrying *KIF3A* gene using Lipofectamine (Lipo) 2000 (Invitrogen, USA) to interfere or increase the expression of *KIF3A*. The sequences (5'-3') of Kif3a siRNAs were: Kif3a siRNA1, GGCCAGC AGAUUACAAUAdTdT; Kif3a siRNA2, GCCAGCAGAUUACAAUUA UdTdT. The cells transfected with scramble siRNA or empty AdV were served as negative control. Transfection was conducted according to the manufacturer's recommended procedure. The siRNA and *KIF3A*-expressing construct were supplied by Sangon Biotech Co., Ltd (Shanghai, China). After 48 h of the transfection, the cells were stimulated with IL-4, IL-13 and TNF- α (all at 10 ng/ml) [14,15]. After 24 h, apoptosis was determined using flow cytometry. The mRNA expression of CCL17, CCL26, IL-5 and IL-8, and protein expression of β -catenin and COX-2 were measured by qRT-PCR and western blotting respectively.

2.8. Detection of apoptosis by flow cytometry

After incubation with IL-4, IL-13 and TNF- α , the cells were harvested and resuspended in 400 μ l of $1 \times$ binding buffer solution with a concentration of 1×10^6 cells/ml. Then the cells were stained with 1 μ g/ml of annexin V-FITC and 2.5 μ g/ml of PI. After incubation for 15 min at room temperature in the dark, the fluorescence was determined by a BD FACSCalibur (BD Biosciences, San Jose, CA, USA) and the flow cytometry data were analyzed using the FlowJo Software (Version 7.6.1, Tri Star Inc., Ashland, OR, USA). Apoptotic cells are identified by positivity for Annexin-V.

2.9. qRT-PCR

Total cellular RNA was prepared from selected tissues and cultured cells using TRIzol Reagent (Invitrogen) according to the manufacturer's instruction. First-strand cDNA was synthesized using the Superscript First-Strand Kit (Invitrogen). Quantitative real-time PCR (qRT-PCR) was carried out with iQ SYBR Green Supermix (Bio-Rad) using primers: KIF3A (mouse), forward primer 5'-GAGGAAGGGGAAGAAGTGTCAG-3', reverse primer 5'-TCCACCATCTTATCTGGGAA A-3'; COX-2 (mouse), forward primer 5'-GGGCCATGGAGTGGACTTAAA-3', reverse primer 5'-ACTCTGTGTGCTCCGAAG-3'; IL-4 (mouse), forward primer 5'-CCATATCCACGGATGC GACA-3', reverse primer 5'-CTGTGGTGTTCTTCGTTGCTG-3'; IL-5 (mouse), forward primer 5'-TGGGGGTACTGTGGAATGC-3', reverse primer 5'-CCACACTTCTCTTTTGGCCG-3'; IL-13 (mouse), forward primer 5'-TCACACAAGACCAGACTCCCC-3', reverse primer 5'-CTCC TCATTAGAAGGGGCCG-3'; IL-6 (mouse), forward primer 5'-CCAAGAGATAAGCTGGAGT CACA-3', reverse primer 5'-CGCACTAGGTTTGCCGAGTA-3'; IL-10 (mouse), forward primer 5'-GGCGCTGTCATCGATTCTC-3', reverse primer 5'-ATGGCCTGTAGACACCTGG-3'; TNF- α (mouse), forward primer 5'-AGGCACTCC CCAAAAGATG-3', reverse primer 5'-CCAC TTGGTGGTTGTGAGTG-3'; CCL17 (human), forward primer 5'-CCAGGGATGCCATCGTT TTG-3', reverse primer 5'-TAGTCCCGGGAGACAGTCAG-3'; CCL26 (human), forward primer 5'-TTGAGGCTGAGCCAAA GACC-3', reverse primer 5'-TGGTAGTGAATATCACAG CCG-3'; IL-5 (human), forward primer 5'-AGCCAATGAGACTCTGAGGAT-3', reverse primer 5'-TTTCCACAGTAC CCCCTTGC-3'; IL-8 (human), forward primer 5'-GCTCTGTGTGAAGGT GCAGTT-3', reverse primer 5'-ACCCAGTTTTCCTTGGGGTC-3'. Samples were analyzed in duplicate. Quantitation of gene expression was calculated by the comparative Ct method and was normalized by GAPDH expression in each sample.

2.10. Western blotting

The tissues and cells were lysed in ice-cold RIPA lysis buffer (Beyotime Inc., Nanjing, China) and nuclear extracts were collected by using a nuclear extraction kit (Millipore, Billerica, USA). The total protein content was measured using a BCA protein assay kit (Appligen, China). Afterwards, equal total proteins were separated by 10% SDS-PAGE and electrotransferred onto PVDF membranes (Thermo Fisher Scientific, USA). After blocking for 30 min at room temp with 5% non-fat milk, the membranes were incubated overnight at 4 °C with primary antibodies. After several washes, the membranes were incubated with an appropriate HRP-conjugated secondary antibody for 1 h at room temp. Protein bands were visualized using the ECL kits (Amersham) and ImageJ software was used for densitometry analysis of western blotting. The antibodies used were listed as below: KIF3A (ab133587, 1:1000, Abcam), Caspase 3 (9664, 1:1000, Cell Signaling Technology), RAD50 (ab124682, 1: 1000, Abcam), COX-2 (ab62331, 1: 1000, Abcam), β -catenin (ab32572, 1: 1000, Abcam) and GAPDH (ab8245, 1: 500, Abcam).

2.11. Statistical analysis

The data are shown as mean $\pm$ SD from at least three experiments. Statistical evaluation of the results was performed using Student's *t*-test, and *P* values of less than 0.05 or 0.01 were considered statistically significant.

3. Results

3.1. Reduced KIF3A in epithelia of nasal mucosa and bronchia of asthmatic mice was alleviated by KIF3A adenovirus (AdV)

KIF3A is a motor subunit of kinesin-2, which is composed of two kinesin related subunits (KIF3A and KIF3 B or 3C) and a binding protein (KAP3) and plays an important role in generation and mechanism of cilia [16,17]. Gene expression profile analysis showed that the expression of KIF3A gene was significantly decreased in children with asthma and asthma model, suggesting that the downregulation of KIF3A may contribute to the pathogenesis of asthma [8]. In accordance with previous reports, the present work showed that the transcription and translation of KIF3A were significantly decreased in the epithelia of nasal mucosa and bronchia from mice with OVA induced asthma compared to saline-treated controls, evidenced by qRT-PCR, western blotting and immunohistochemical analysis (Fig. 1). To explore the effect of impaired KIF3A gene expression on the structural integrity of epithelium, stable overexpression of KIF3A in asthmatic mice was carried out through nasal infection of adenovirus (AdV) carrying mouse KIF3A gene. The results showed KIF3A expression was significantly increased by KIF3A adenovirus transfection, even higher than normal level (Fig. 1).

3.2. Increased epithelia apoptosis of nasal mucosa and bronchia in asthma was suppressed by KIF3A overexpression

It was found that KIF3A knockdown in renal tubular epithelial cells led to a decrease in cilia and an increase in cisplatin induced apoptosis [10]. In addition, KIF3A knockdown also promoted apoptosis in photoreceptor cell [11]. Combined with evidence that KIF3A overexpression observably suppressed epithelium exfoliation in asthma, we speculated that insufficient expression of KIF3A in epithelial cells contributed to its sensitivity to apoptosis in asthma. To address this, apoptosis in nasal mucosa and bronchia epithelium was evaluated by TUNEL staining and determination of cleaved caspase-3 through western blotting assay. The results of TUNEL staining revealed that overexpression of KIF3A significantly reduced the increased TUNEL positive cells in both nasal mucosa and bronchia epithelium from asthmatic mice (Fig. 2AB). The results of western blotting analysis showed the increased cleaved caspase-3 (Casp 3 p17) levels was also decreased by KIF3A overexpression (Fig. 2CD). DNA repair protein RAD50, also known as RAD50, is a protein that is encoded by the RAD50 gene located closely to KIF3A on the chromosome. RAD50 is of great importance to homologous recombination and DNA double-strand break repair and mice carrying a mutation in RAD50 showed increased apoptosis [18]. This work showed the protein expression of RAD50 was also downregulated in nasal mucosa and bronchia epithelium isolated from mice with asthma, which was reversed by KIF3A overexpression (Fig. 2C/E). Taken together, the data suggested KIF3A overexpression could alleviate epithelia apoptosis of nasal mucosa and bronchia in asthma.

3.3. Bronchial inflammation in asthmatic mice was suppressed by KIF3A overexpression

Extensive research has demonstrated the significant role of CD4 Th2 immune response and its associated cytokines (e.g. IL-4, IL-5, and IL-13)

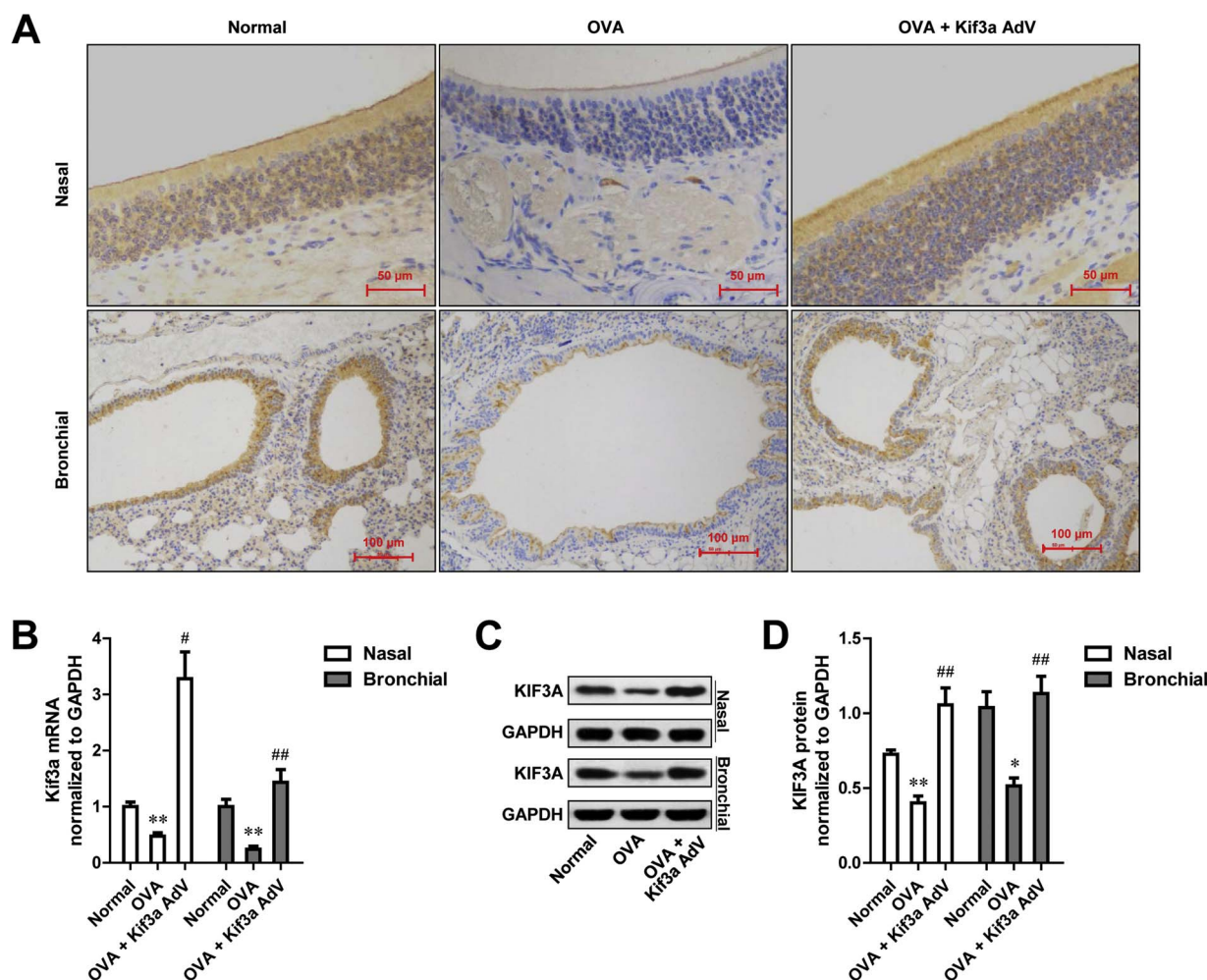


Fig. 1. Changes of *KIF3A* expression and tissue morphology in epithelia of nasal mucosa and bronchia of mice treated with OVA. A–D. *KIF3A* expression was determined using immunohistochemistry, qRT-PCR and western blotting. AdV: adenovirus; * $P < 0.05$, ** $P < 0.01$, compared with Normal group; # $P < 0.05$, ## $P < 0.01$, compared with OVA treatment group.

in the pathogenesis of allergic asthma. IL-6, like other inflammatory cytokines, has been shown to be elevated in asthmatic subjects compared with healthy controls [19], and play an active role in pathogenesis of asthma [20]. COX-2, an important pro-inflammatory mediator, is undetectable in most tissues under normal condition, and has been shown to be increased in bronchial asthma [21]. Although *KIF3A* does not encode Th2 cytokines, but it is a nearby gene of the Th2 region, which indicated that impaired expression of *KIF3A* may contribute to the occurrence and development of inflammatory diseases, including asthma. A recent research showed *KIF3A* deficiency in mice resulted in increased airway hyper-responsiveness and Th2-mediated, eosinophilic inflammation [7]. The present work showed that overexpression of *KIF3A* could efficiently decrease the upregulated mRNA expression of COX-2, Th2 cytokines (IL-4, IL-5 and IL-13), and proinflammatory cytokines (IL-6 and TNF- α), whereas increase the decreased expression of anti-inflammatory cytokine IL-10 in lungs of asthmatic mice (Fig. 3). The data suggested impaired *KIF3A* expression may account for the bronchial inflammation in asthma.

3.4. Aberrant *KIF3A* expression influences the survival and cytokine expression of human bronchial epithelial cell line 16HBE 14o-

To explore the influence of bronchial inflammation on bronchial epithelial cell and the role of *KIF3A* gene played in this process, human bronchial epithelial cell line 16HBE 14o- were stimulated with IL-4, IL-13 and TNF- α , accompanied by *KIF3A* knockdown or overexpression by

using siRNA targeting *KIF3A* or *KIF3A* AdV respectively. Apoptosis, mRNA expression of CCL17, CCL26, IL-5 and IL-8, and protein expression of COX-2 and β -catenin were determined using flow cytometry, qRT-PCR and western blotting. The results showed that under co-simulation of IL-4, IL-13 and TNF- α , *KIF3A* knockdown significantly promoted epithelium apoptosis, whereas overexpression of *KIF3A* exhibited the opposite effect (Fig. 4A,B). In addition, *KIF3A* knockdown markedly facilitated the transcription of CCL17, CCL26, IL-5 and IL-8, and increased the protein levels of COX-2 and β -catenin (nucleus). As expected, *KIF3A* overexpression decreased the expression of CCL17, CCL26, IL-5, IL-8, COX-2 and β -catenin in bronchial epithelial cells (Fig. 4C–E).

4. Discussion

In this work, we found that *KIF3A* expression was significantly decreased in epithelia of nasal mucosa and bronchia of mice with ovalbumin (OVA) induced asthma, and which was significantly associated with the pathologic features of asthma. Overexpression of *KIF3A* could efficiently suppress the increased epithelia apoptosis of nasal mucosa and bronchia and bronchial inflammation. *In vitro*, *KIF3A* knockdown increased the susceptibility of human bronchial epithelial cell line 16HBE 14o- to TNF- α induced apoptosis and the expression of CCL17, CCL26, IL-5 and IL-8, which may resulted from increased production of COX-2 and β -catenin translocation.

KIF3A gene encodes one subunit of the heterotrimeric motor

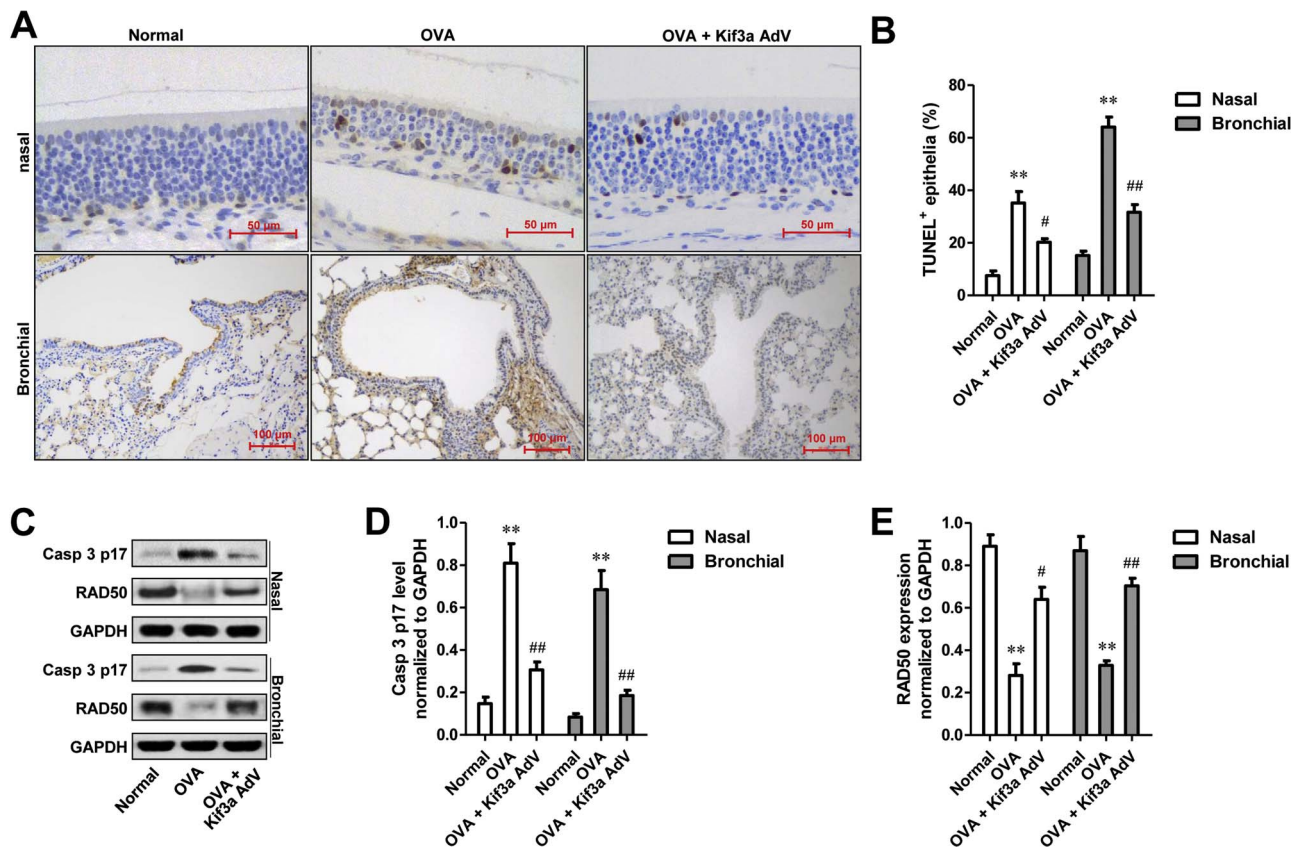


Fig. 2. Apoptosis in nasal mucosa and bronchial epithelium measured by TUNEL staining and western blotting after OVA induced asthma and *KIF3A* adenovirus infection. A. TUNEL staining of epithelia of nasal mucosa and bronchia. B. The percentage of TUNEL positive cells. C–E. Protein levels of activated caspase 3 fragment p17 and DNA repair protein RAD50 in nasal mucosa and bronchial tissues. * $P < 0.05$, ** $P < 0.01$, compared with Normal group; # $P < 0.05$, ## $P < 0.01$, compared with OVA treatment group.

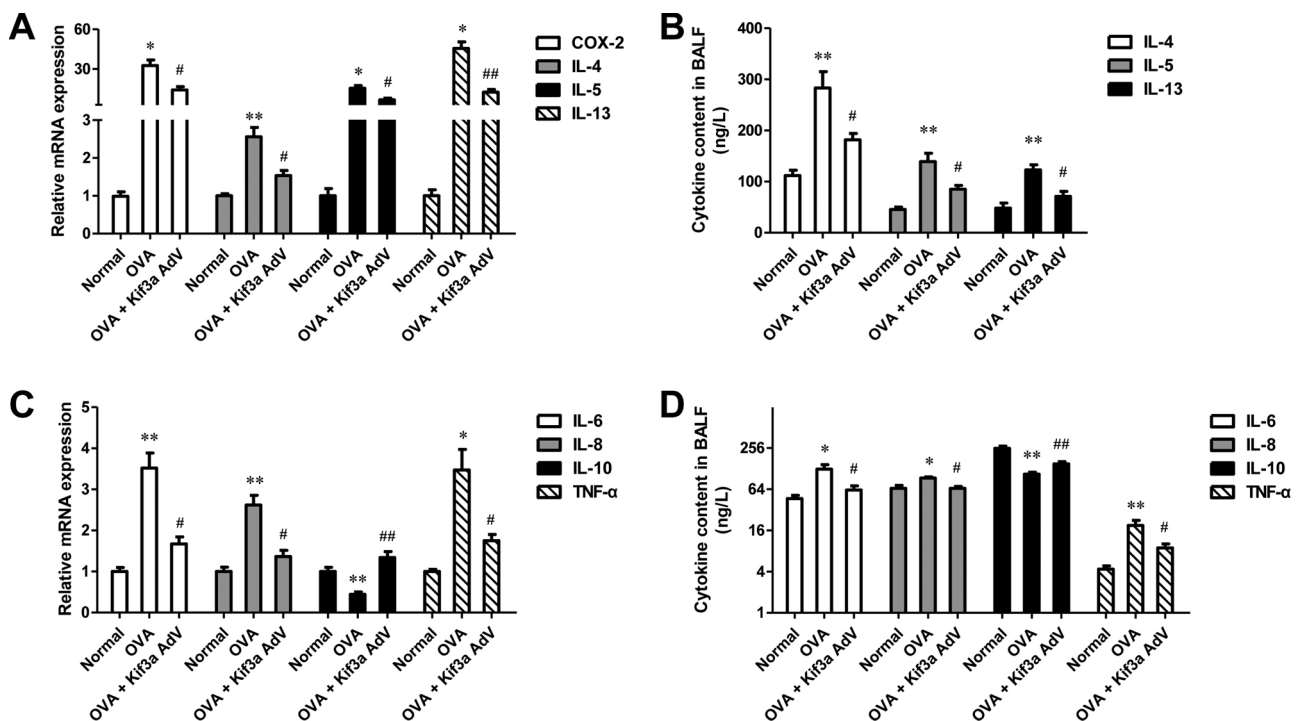


Fig. 3. The expression of inflammation associated genes (COX-2, IL-4, IL-5, IL-13, IL-6, IL-8, IL-10 and TNF-α) at mRNA level in lungs from each group. A. COX-2 and Th2 cell cytokines (IL-4, IL-5 and IL-13) mRNA levels measured with qRT-PCR; B. The secretion levels of Th2 cell cytokines in broncho alveolar lavage fluid (BALF) determined with ELISA assay; C–D mRNA levels of proinflammatory cytokines (IL-6, IL-8 and TNF-α) and anti-inflammatory cytokine IL-8 measured with qRT-PCR, and their secretion levels in BALF measured with ELISA assay. * $P < 0.05$, ** $P < 0.01$, compared with Normal group; # $P < 0.05$, ## $P < 0.01$, compared with OVA treatment group.

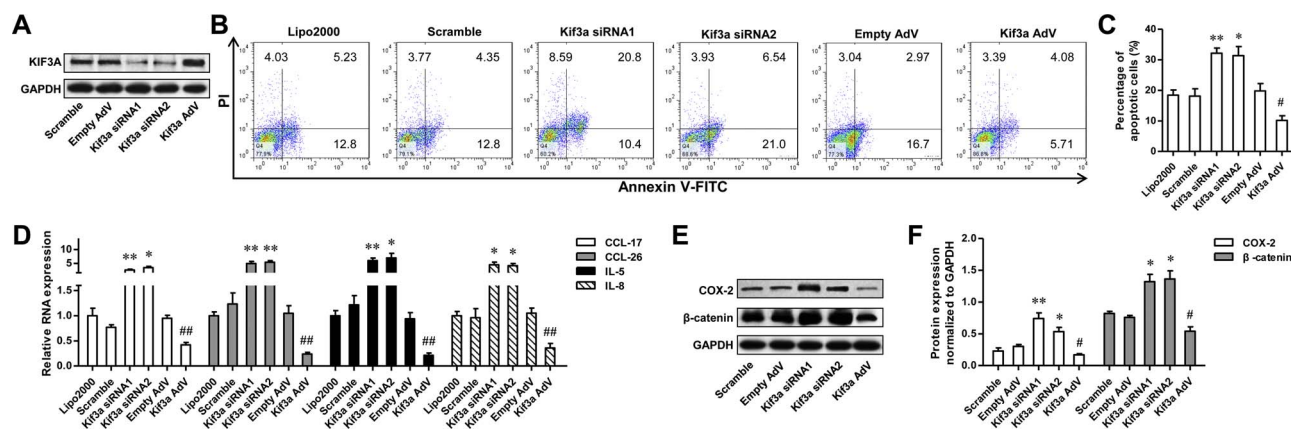


Fig. 4. The effects of Kif3a knockdown and overexpression on apoptosis and cytokine expression of 16HBE 14o- cells. **A.** Knockdown or overexpression effect of Kif3a siRNAs or Kif3a AdV on the protein expression of KIF3A in 16HBE 14o- cells. **B–C.** Apoptosis of 16HBE 14o- cells before and after the knockdown or overexpression of Kif3a. **D.** Cytokines (chemokines CCL17 and CCL26 and inflammatory factors IL-5 and IL-8) expressed in 16HBE 14o- cells measured with qRT-PCR. **E–F.** COX-2 and β -catenin protein levels measured with western blotting. * $P < 0.05$, ** $P < 0.01$, compared with Scramble group (transfected with scramble siRNA, served as negative control); # $P < 0.05$, ## $P < 0.01$, compared with empty AdV group (infected with empty AdV).

protein, kinesin-2, which consists of two kinesin-related subunits (called KIF3A and KIF3B or 3C in vertebrates) and an associated protein (KAP3), and it transports protein complexes, nucleic acids and organelles towards the “plus” ends of microtubule tracks within cells. Downregulation of *KIF3A* has been observed in asthmatic child patients and mice by different researchers [7,8]. However, investigations about its role in bronchial inflammation are rare. Our findings share broad similarities with an earlier study by Vummidi Giridhar and colleagues who found that *KIF3A* deficient in mice resulted in increased airway hyper-responsiveness and Th2-mediated, eosinophilic inflammation [7,22]. However, the exact mechanism is not well understood. The ciliated epithelium that covers the surface of the airways forms an immunologically active natural barrier to invasion and injury by inhaled pathogenic organisms and particulate material. Impaired *KIF3A* expression in asthma may influence the generation and mechanism of cilia, and ciliary dysfunction causes impaired mucociliary clearance and increased susceptibility to infection, which contribute to the pathogenesis of asthma [23]. In addition, *KIF3A* also play important roles in microtubule assembly and intracellular transport of multiple protein cargos, and disrupted microtubular transport within the ECs may influence the increased Th2 responses [22]. Moreover, *KIF3A* gene is located on human chromosomal region 5q31-33 contiguously with the IL-4/IL-13 genes [24,25], which implied the *KIF3A* alleles in the pathogenesis of asthma.

To the best of our knowledge, no data exist concerning the influence of *KIF3A* on airway epithelial cells (ECs) apoptosis. In this work, we found that decreased *KIF3A* in human bronchial epithelial cell line 16HBE 14o- significantly increased its susceptibility to TNF- α induced apoptosis. The facilitating effect of impaired *KIF3A* expression on apoptosis has been observed in various cell types; however the corresponding mechanism has not been fully understood. David A. Cano et al. found tissue-specific loss of *KIF3A* in mice resulted in increased acinar cell apoptosis evidenced by TUNEL staining [26]. A study by Joseph R. Marszalek et al. found remove of *KIF3A* using Cre-loxP mutagenesis from photoreceptors caused the accumulations of opsin, arrestin, and membranes within the photoreceptor inner segment, and eventually apoptotic death [11]. Lopes et al. found the mislocalization of opsin is a major cause of *KIF3A*-null photoreceptor cell death, and that the cell death follows a typical apoptotic pathway [27]. Shixuan Wang et al. found *KIF3A* knockdown in cultured human proximal tubular HK-2 epithelial cells led to suppressed cilia and sensitized HK-cells to cisplatin-induced caspase dependent apoptosis, which was abolished by U0126, a specific pharmacological inhibitor of MEK that blocks ERK1/2 phosphorylation and activation [28]. In addition,

Joseph E. Aslan and Gary Thomas suggested that apoptotic paranuclear clustering of mitochondria may also account for *KIF3A* deficiency induced apoptosis [29]. However, the exact mechanisms for increased susceptibility to TNF- α induced apoptosis in *KIF3A* knockdown bronchial ECs remains to be further explored and resolved.

In addition to promote apoptosis, *KIF3A* knockdown also facilitated the generation of chemokines CCL17 and CCL26 and inflammatory factors IL-5 and IL-8 in human bronchial epithelial cell line 16HBE 14o- under the stimulation of IL-4, IL-13 and TNF- α . CCL17 is a Th2-specific chemokine and has been highly implicated in the pathogenesis of bronchial asthma [30]. CCL17 binds and induces chemotaxis in Th2 cells by specifically and exclusively interacting with the CC chemokine receptor 4 (CCR4) that is selectively expressed on Th2 cells. It has been shown that CCL17 protein expression is up-regulated in the airway epithelium of mice with asthma [31], and the serum levels of CCL17 were also significantly increased in asthmatic patients compared with healthy individuals [32]. More recently, administration of CCR4 blocking antibody abolished the airway eosinophilia, goblet cell hyperplasia, IgE synthesis and bronchial hyperreactivity in a humanized severe combined immunodeficiency model of asthma [33]. CCL26 is chemotactic for eosinophils and basophils recruitment to asthmatic airways by binding to the cell surface chemokine receptor CCR3 [34,35], and have been detected in bronchoalveolar lavage of asthmatics [36]. Recent reports indicate that bronchial epithelial cells had increased levels of CCL26 when stimulated by IL-4 and IL-13 [37]. IL-5 is an eosinophil chemotactic factor, and highly purified murine IL-5 stimulates eosinophil function and prolongs *in vitro* survival [38]. IL-5 was constitutively expressed by both transformed human bronchial epithelial cell lines (A549 and 16HBE14o-) and primary human bronchial and nasal epithelial cells grown in culture, and was upregulated by TNF- α stimulation [39]. IL-8 is a neutrophil chemotactic factor, and IL-8 released by bronchial ECs is thought to be of particular importance in attracting neutrophils and monocytes to sites of inflammation [40]. Stríz et al. showed Th2 cytokine IL-4 and IL-13 could stimulate human bronchial ECs to release IL-8 [40].

To the best of our knowledge, no relation has been established between *KIF3A* and the mRNA expression of CCL17, CCL26, IL-5 and IL-8 in the ECs. We found that COX-2 generation and β -catenin translocation were significantly increased after *KIF3A* knockdown in human bronchial epithelial cell line 16HBE 14o-, which may account for the increased expression of these chemokines and inflammatory factors. It has been documented that *KIF3A* constrains β -catenin-dependent Wnt signaling through dual ciliary and non-ciliary mechanisms [41], and the cyst epithelial cells in *KIF3A* knockout mice exhibit increased

expression and translocation of β -catenin [42]. In addition, β -catenin stabilization in the cilia cells isolated from *KIF3A* $-/-$ mice have been observed. This stabilization may results in NF- κ B activation [41], which also plays a crucial role in immune responses, resulting in the activation of target effectors, such as COX-2 [43]. Thus, we speculated that decreased expression of *KIF3A* in bronchial ECs may modulate the expression of CCL17, CCL26, IL-5 and IL-8 through Wnt/ β -catenin pathway and NF- κ B activation, which need further validation and support.

5. Conclusion

KIF3A expression is significantly reduced in epithelia of nasal mucosa and bronchia of asthmatic mice, which was tightly related to epithelial cell apoptosis and bronchitis inflammation. *KIF3A* may be a potential target for asthma treatment.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgement

This study was sponsored by Chongqing Association for Science and Technology (Grant No.: KJXX2017016).

References

- [1] S. Croisant, Epidemiology of Asthma: Prevalence and Burden of Disease, Springer, US, 2014.
- [2] J.V. Fahy, Type 2 inflammation in asthma—present in most, absent in many, Nat. Rev. Immunol. 15 (2015) 57.
- [3] H.J. Ryu, H.Y. Jung, J.S. Park, G.M. Ryu, J.Y. Heo, J.J. Kim, et al., Gene-based single nucleotide polymorphisms and linkage disequilibrium patterns of 29 asthma candidate genes in the chromosome 5q31-33 region in Koreans, Int. Arch. Allergy Immunol. 139 (2006) 209–216.
- [4] J.W. Holloway, C. Lonjou, B. Beghé, Q. Peng, T.R. Gaunt, I. Gomes, et al., Linkage analysis of the 5q31-33 candidate region for asthma in 240 UK families, Genes Immun. 2 (2001) 20–24.
- [5] P. Kauppi, K. Lindblad-Toh, P. Sevon, H.T.T. Toivonen, J.D. Rioux, A. Villapakkam, et al., A Second-Generation Association Study of the 5q31 cytokine gene cluster and the Interleukin-4 receptor in asthma, Genomics 77 (2001) 35–42.
- [6] J. Donfack, D.H. Schneider, Z. Tan, T. Kurz, I. Dubchak, K.A. Frazer, et al., Variation in conserved non-coding sequences on chromosome 5q and susceptibility to asthma and atopy, Respir. Res. 6 (2005) 1–12.
- [7] P.V. Giridhar, S.M. Bell, A. Sridharan, J.B. Myers, H. Johansson, M. Lisa, et al., *KIF3A* regulates lung morphogenesis, airway epithelial cell repair and inflammation. Airway epithelium: molecular regulation of inflammation, Am. Thor. Soc. 193 (2016) A4654–A4654.
- [8] M.B. Kovacic, J.M. Myers, N. Wang, L.J. Martin, M. Lindsey, M.B. Ericksen, et al., Identification of *KIF3A* as a novel candidate gene for childhood asthma using RNA expression and population allelic frequencies differences, PLoS One 6 (2011) 449–453.
- [9] A. Trautmann, P. Schmid-Grendelmeier, K. Krüger, R. Cramer, M. Akdis, A. Akkaya, et al., T cells and eosinophils cooperate in the induction of bronchial epithelial cell apoptosis in asthma, J. Allergy Clin. Immunol. 109 (2002) 329–337.
- [10] S. Wang, Q. Wei, G. Dong, D. Zheng, ERK-mediated suppression of cilia in cisplatin-induced tubular cell apoptosis and acute kidney injury, Biochim. Biophys. Acta 1832 (2013) 1582–1590.
- [11] J.R. Marszalek, X. Liu, E.A. Roberts, D. Chui, J.D. Marth, D.S. Williams, et al., Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors, Cell 102 (2000) 175–187.
- [12] C. Yang, J. Li, H. Lin, K. Zhao, C. Zheng, Nasal mucosa derived-mesenchymal stem cells from mice reduce inflammation via modulating immune responses, PLoS One 10 (2015) e0118849.
- [13] L. Karra, O. Haworth, R. Priluck, B.D. Levy, F. Levischaffer, Lipoxin B4 promotes the resolution of allergic inflammation in the upper and lower airways of mice, Mucosal Immunol. 8 (2014) 852–862.
- [14] A. Trautmann, K. Krüger, M. Akdis, D. Müller-Wening, A. Akkaya, E.-B. Bröcker, et al., Apoptosis and loss of adhesion of bronchial epithelial cells in asthma, Int. Arch. Allergy Immunol. 138 (2005) 142–150.
- [15] I. Striž, T. Mio, Y. Adachi, R. Robbins, D. Romberger, S. Rennard, IL-4 and IL-13 stimulate human bronchial epithelial cells to release IL-8, Inflammation 23 (1999) 545–555.
- [16] J.R. Marszalek, L.S.B. Goldstein: Situs Inversus, Embryonic ciliary morphogenesis defects in mouse mutants lacking the *KIF3A* subunit of Kinesin-II, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 5043–5048.
- [17] K.C. Corbit, A.E. Shyer, W.E. Dowdle, J. Gaulden, V. Singla, J.F. Reiter, *Kif3a* constrains β -catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms, Nat. Cell Biol. 10 (2008) 70–76.
- [18] C.F. Bender, M.L. Sikes, R. Sullivan, L.E. Huye, B.M. Le, D.B. Roth, et al., Cancer predisposition and hematopoietic failure in Rad50S/S mice, Genes. Dev. 16 (2002) 2237–2251.
- [19] W.A. Neveu, J.L. Allard, D.M. Raymond, L.M. Bourassa, S.M. Burns, J.Y. Bunn, et al., Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function, Respir. Res. 11 (2010) 28.
- [20] M. Rincon, C.G. Irvin, Role of IL-6 in asthma and other inflammatory pulmonary diseases, Int. J. Biol. Sci. 8 (2011) 1281–1290.
- [21] R. Taha, R. Olivenstein, T. Utsumi, P. Ernst, P.J. Barnes, I.W. Rodger, et al., Prostaglandin H synthase 2 expression in airway cells from patients with asthma and chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 161 (2000) 636–640.
- [22] P.V. Giridhar, S.M. Bell, A. Sridharan, P. Rajavelu, J.A. Kitzmiller, C.L. Na, et al., Airway epithelial *KIF3A* regulates Th2 responses to aeroallergens, J. Immunol. 197 (2016) 4228–4239.
- [23] B. Thomas, A. Rutman, R.A. Hirst, P. Haldar, A.J. Wardlaw, J. Bankart, et al., Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma, J. Allergy Clin. Immunol. 126 (2010) 722–729 e2.
- [24] D.J. Cousins, T.H. Lee, D.Z. Staynov, Cytokine coexpression during human TH1/TH2 cell differentiation: direct evidence for coordinated expression of th2 cytokines, J. Immunol. 169 (2002) 2498–2506.
- [25] K.A. Frazer, Y. Ueda, Y. Zhu, V.R. Gifford, M.R. Garofalo, N. Mohandas, et al., Computational and biological analysis of 680 kb of DNA sequence from the human 5q31 cytokine gene cluster region, Genome Res. 7 (1997) 495–512.
- [26] D.A. Cano, S. Sekine, M. Hebrok, Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis, Gastroenterology 131 (2006) 1856–1869.
- [27] V.S. Lopes, D. Jimeno, K. Khanobdee, X. Song, B. Chen, S. Nusinowitz, et al., Rapid rod photoreceptor cell death due to Kinesin-2 dysfunction is dependent on apoptosis triggered by mislocalized opsin, Invest. Ophthalmol. Vis. Sci. 51 (2010) 5809–5809.
- [28] S. Wang, Q. Wei, G. Dong, Z. Dong, ERK-mediated suppression of cilia in cisplatin-induced tubular cell apoptosis and acute kidney injury, Biochim. Biophys. Acta 1832 (2013) 1582–1590.
- [29] J.E. Aslan, G. Thomas, Death by committee: organellar trafficking and communication in apoptosis, Traffic 10 (2009) 1390–1404.
- [30] T. Sekiya, Y. Tsunemi, M. Miyamasu, K. Ohta, A. Morita, H. Saeki, et al., Variations in the human Th2-specific chemokine TARC gene, Immunogenetics 54 (2003) 742–745.
- [31] T. Sekiya, M. Miyamasu, M. Imanishi, H. Yamada, T. Nakajima, M. Yamaguchi, et al., Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells, J. Immunol. 165 (2000) 2205–2213.
- [32] T. Sekiya, H. Yamada, M. Yamaguchi, K. Yamamoto, A. Ishii, O. Yoshie, et al., Increased levels of a Th2-type CC chemokine thymus and activation-regulated chemokine (TARC) in serum and induced sputum of asthmatics, Allergy 57 (2002) 173–177.
- [33] F. Perros, H.C. Hoogsteden, A.J. Coyle, B.N. Lambrecht, H. Hammad, Blockade of CCR4 in a humanized model of asthma reveals a critical role for DC-derived CCL17 and CCL22 in attracting Th2 cells and inducing airway inflammation, Allergy 64 (2009) 995–1002.
- [34] N. Berkman, S. Ohnnona, F.K. Chung, R. Breuer, Eotaxin-3 but not eotaxin gene expression is upregulated in asthmatics 24 hours after allergen challenge, Am. J. Respir. Cell Mol. Biol. 24 (2001) 682–687.
- [35] V. Provost, M.C. Larose, A. Langlois, M. Rola-Pleszczynski, N. Flamand, M. Laviolette, CCL26/eotaxin-3 is more effective to induce the migration of eosinophils of asthmatics than CCL11/eotaxin-1 and CCL24/eotaxin-2, J. Leukoc. Biol. 94 (2013) 213–222.
- [36] B.O. Abonyo, M.S. Alexander, A.S. Heiman, Autoregulation of CCL26 synthesis and secretion in A549 cells: a possible mechanism by which alveolar epithelial cells modulate airway inflammation, AJP Lung Cell. Mol. Phys. 289 (2005) 478–488.
- [37] A. Komiya, H. Nagase, H. Yamada, T. Sekiya, M. Yamaguchi, Y. Sano, et al., Concerted expression of eotaxin-1 eotaxin-2, and eotaxin-3 in human bronchial epithelial cells, Cell. Immunol. 225 (2003) 91–100.
- [38] Y. Yamaguchi, Y. Hayashi, Y. Sugama, Y. Miura, T. Kasahara, S. Kitamura, et al., Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor, J. Exp. Med. 167 (1988) 1737–1742.
- [39] S. Salvi, A. Semper, A. Blomberg, J. Holloway, Z. Jaffar, A. Papi, et al., Interleukin-5 production by human airway epithelial cells, Am. J. Respir. Cell Mol. Biol. 20 (1999) 984–991.
- [40] I. Striz, T. Mio, Y. Adachi, R.A. Robbins, D.J. Romberger, S.I. Rennard, IL-4 and IL-13 stimulate human bronchial epithelial cells to release IL-8, Inflammation 23 (1999) 545–555.
- [41] K.C. Corbit, A.E. Shyer, W.E. Dowdle, J. Gaulden, V. Singla, M.H. Chen, et al., *Kif3a* constrains beta-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms, Nat. Cell Biol. 10 (2008) 70–76.
- [42] F. Lin, T. Hiesberger, K. Cordes, A.M. Sinclair, L.S.B. Goldstein, S. Somlo, et al., Kidney-specific inactivation of the *KIF3A* subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 5286–5291.
- [43] S. Amit, Y. Ben-Neriah, NF-kappaB activation in cancer: a challenge for ubiquitination- and proteasome-based therapeutic approach, Semin. Cancer Biol. 13 (2003) 15–28.