

Clinical efficacy of recombinant human latrophilin 3 antibody in the treatment of pediatric asthma

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Abstract. Pediatric asthma is a chronic pulmonary inflammatory disease featuring hypersecretion of mucus and inflammation in the airway, resulting in dysfunction of the airway smooth muscle. Previous evidence demonstrated that latrophilins, a novel family of receptors, present a beneficial effect on airway smooth muscle cells. In the present study, the therapeutic effects of recombinant human latrophilin 3 (rhLPHN3) antibody (Ab) in patients with pediatric asthma were investigated, and the molecular mechanism underlying the function of LPHN3 in the treatment of asthma in clinical practice was examined. A total of 342 pediatric asthma cases were recruited and randomly divided into three groups, receiving treatment with rhLPHN3 Ab (n=134), salbutamol (n=108) or montelukast (n=100) by nasal aerosolization. Each group received the respective clinically tested dose for 16 weeks. Inflammatory factors interleukin (IL)-10, IL-17, IL-4, matrix metalloproteinase-9 (MMP-9), interferon- γ (IFN- γ) and transforming growth factor- β (TGF- β) levels in peripheral blood mononuclear cells were analyzed prior to and post treatment. The clinical outcomes revealed that pathological alterations were significantly improved following treatment with rhLPHN3 Ab for patients with pediatric asthma when compared with those receiving salbutamol and montelukast. It was also observed that rhLPHN3 Ab downregulated the plasma concentration levels of IL-10, IL-17, IL-4 and MMP-9, and upregulated IFN- γ and TGF- β levels in the three groups. In addition, clinical data demonstrated that rhLPHN3 Ab significantly promoted E-selectin and mucin 5AC expression, as well as improved the activation of nuclear factor (NF)- κ B p65 DNA binding activity and the phosphorylation levels of protein kinase A. Furthermore, rhLPHN3 Ab markedly improved adhesion and proliferation of airway smooth muscle cells, which led to promotion of the contraction of these cells.

In conclusion, these clinical data suggest that rhLPHN3 Ab serves an important role in the inhibition of inflammatory mediators through downregulation of NF- κ B signaling pathway, which contributes to airway remodeling and bronchodilation in patients with pediatric asthma.

Introduction

Asthma is a chronic inflammatory disorder that affects >300 million people annually worldwide (1), and the worldwide incidence of pediatric asthma is currently increasing. Asthma is known to be a heterogeneous pulmonary disease characterized by chronic airway inflammation, leading to respiratory swelling and dyspnea (2-4). It has been demonstrated that mast cells, eosinophil granulocytes, cytokines and T cells serve essential roles in the initiation and progression of pediatric asthma (5,6). Previous studies have also indicated that the inflammatory factors matrix metalloproteinase-9 (MMP-9) and transforming growth factor- β (TGF- β) induced by immunocytes are closely correlated with airway inflammation and airway remodeling (7,8).

In recent years, several studies have observed that the incidence of pediatric asthma presents an increasing trend according to clinical investigation (9,10). Pediatric asthma was reported as the most common chronic respiratory disease in children, and this disease deleteriously impacts all aspects of systemic function (11). In addition, it has been suggested that patients with pediatric asthma exhibit behavioral and psychological problems that contribute to the risk for functional impairments and communication difficulties (12). Furthermore, environmental factors have been regarded as one of the main preventable contributors for the epidemiology of pediatric asthma (13). Notably, although various treatments have been proposed for pediatric asthma, numerous patients remain refractory under current clinical therapies. Therefore, novel and efficient treatments need to be urgently identified for patients with pediatric asthma.

Latrophilin agents, a novel family of receptors, have been reported to present beneficial effects on airway smooth muscle cells by regulating airway smooth muscle cell adhesion, proliferation and contraction (14). However, their physiological function remains unclear (15). Therefore, the present clinical trial hypothesized that recombinant human latrophilin 3 antibody (rhLPHN3Ab) may exert a beneficial effect on the

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recovery of patients with pediatric asthma due to its efficacy on the improvement of airway smooth muscle cell contraction, determined by forced expiratory volume (FEV), forced vital capacity (FVC) and peak expiratory flow (PEF).

Several signaling pathways involved in the initiation and development of pediatric asthma have been proposed, including c-Jun N-terminal kinase, tumor necrosis factor- α (TNF- α), protein kinase A (PKA)/nuclear factor- κ B (NF- κ B), MAP kinase-interacting serine/threonine-protein kinase 1/eukaryotic initiation factor 4E, extracellular signal-regulated kinase (ERK)1/2 and receptor for advanced glycation end products/ERK pathways (16-18). However, the mechanism(s) of rhLPHN3-mediated signaling pathway has not been reported in previous studies.

In the present study, the efficacy and underlying mechanism of LPHN3 in the treatment of pediatric asthma was investigated. It was observed that rhLPHN3 Ab decreased the expression levels of inflammatory mediators, and contributed to airway remodeling and bronchodilation through the PKA-induced NF- κ B signaling pathway. Notably, the results indicated that rhLPHN3 Ab was associated with increased expression of E-selectin and mucin 5AC (MUC5AC). Given the clinical outcomes of the patients in the current study, rhLPHN3 Ab may be a potential agent for future application in the treatment of pediatric asthma.

Materials and methods

Ethics statement. This phase-I study (no. YSCOHLY-20110812R1) was conducted between August 2011 and June 2015, in strict accordance with the recommendations and guidelines of the Yishui Central Hospital of Linyi (Linyi, China). This study was approved by the ethics committee of Yishui Central Hospital of Linyi. All patients and their guardians were required to review trial protocols and amendments, and provided informed consent prior to participation.

Patients and treatments. A total of 342 children with capillary bronchitis and asthma (age, 4-11-years-old) were recruited into the present study, and the 22-item sinonasal outcome test (SNOT-22) and Lund-Kennedy endoscopy scores (LKES) were used to evaluate the status of pediatric asthma (19). Patients were subjected to rhLPHN3, salbutamol or montelukast once a day in a double-blind trial at Yishui Central Hospital of Linyi. No others clinical syndromes were observed in this analysis. All children were instructed to wash their mouths thoroughly with normal saline before treatment. The children were randomly divided into three groups and received treatment with salbutamol (12 mg/day; Tianjin Lisheng Pharmaceutical Co., Ltd., Tianjin, China), montelukast (12 mg/day; Hangzhou MSD Pharmaceutical Co., Ltd., Hangzhou, China) or rhLPHN3 Ab (1:100 dilution with 0.9% NaCl, 12 mg/day; cat. no. BA7210, Boao Biological Pharmaceutical Co., Ltd., Tianjin, China) by nasal aerosolization for a total of 16 weeks.

Study design. Double-blind investigation was conducted at two time points: At the baseline stage, and at 16 weeks of treatment for patients with pediatric asthma. The patients continued treatment with the dose of salbutamol (12 mg/day),

montelukast (12 mg/day) or rhLPHN3 Ab (12 mg/day) during the maintenance period.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total mRNA from airway smooth muscle cells (cells obtained by primary culture of smooth muscle tissue) was isolated using an mRNeasy Extraction kit (Qiagen, Inc., Valencia, CA, USA). Extracted mRNA (1 μ g) was transcribed into cDNA using a reverse transcription kit (Qiagen, Inc.). The cDNA (10 ng) was used for qPCR using the SYBR Green Master mix system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All the forward and reverse primers for E-selectin and MUC5AC were synthesized by Invitrogen (Thermo Fisher Scientific, Inc.): E-selectin, forward 5'-CATTGGAGAGAAAGGAAAGTGTG-3' and reverse 5'-GCTTGCATGTACGAA GAGGAT-3'; MUC5AC, forward 5'-TCCAAC TACTACCAA GAAGTCAA-3' and reverse 5'-CAAGGAAATAGACGA TAGCCAA-3'; β -actin, forward 5'-GTGGGCGCCCAGGCA CCA-3' and reverse 5'-CTCCTTAATGTACGCACGAT TT-3'. PCR amplification followed preliminary denaturation at 94°C for 2 min, followed by 38 cycles of 95°C for 30 sec, annealing temperature reduced to 62°C for 30 sec and 72°C for 10 min by volume of 20 μ l containing 50 ng of genomic DNA, 200 μ M dNTP, 2.5 units of Taq DNA polymerase, and 200 μ M primers. Relative mRNA expression changes were calculated by $2^{-\Delta\Delta C_q}$ (20).

Western blot analysis. Airway smooth muscle cells were isolated from pediatric asthma patients by primary culture of smooth muscle tissue and homogenized in lysate buffer containing protease-inhibitor (Invitrogen; Thermo Fisher Scientific, Inc.), then centrifuged at 7,104 x g at 4°C for 10 min. Supernatants were collected and the protein concentration was detected using a Bio-Rad protein assay kit (cat. no. 500-0002; Bio-Rad Laboratories, Inc.) Protein (30 μ g) was separated by 10% SDS-PAGE assays followed by transference onto polyvinylidene difluoride membranes. The membranes were blocked in Tris-buffered saline buffer (50 mmol/l NaCl, 10 mmol/l Tris, pH 7.4) containing 5% nonfat milk for 2 h at room temperature as previously described (20). For western blotting, the following rabbit anti-human primary antibodies were used: pPKA (cat. no. 14270-1-AP, 1:1,000, Proteintech) and β -actin (cat. no. 66009-1-Ig, 1:5,000, Proteintech). Primary antibodies were incubated overnight at 4°C. Membranes were then incubated with secondary antibodies (cat. no. 4410, 1:1,000, Cell Signaling Technology) for 24 h at 4°C. The results were visualized using an enhanced chemiluminescence detection system (Thermo Fisher Scientific, Inc.). BandScan 5.0 software (Glyko, Inc., Novato, CA, USA) was used for the quantification of proteins following western blot analysis.

NF- κ B activation. Airway smooth muscle cells were collected and lysed by three freeze-thaw steps in 200 μ l of 0.25 M Tris-HCl (pH 7.9) plus 1 mM dithiothreitol. Cell extracts were clarified in a microcentrifuge (7,104 x g), and 20 μ l of each extract was incubated with 350 μ l of reaction buffer A [25 mM glycyl-glycine (pH 7.8), 5 mM ATP (pH 7.5), 4 mM EGTA (pH 8.0), 15 mM MgSO₄] and then mixed with 100 μ l 0.25 mM luciferin (Sigma-Aldrich; Merck KGaA) in reaction buffer A. A TD-20/20 luminometer (Turner Designs, Sunnyvale, CA,

Table I. Characteristics of study population.

Characteristic	Salbutamol	Montelukast	rhLPHN3 Ab
No. of patients, n (%)	134 (39.2)	108 (31.6)	100 (29.2)
Gender, n			
Male	60	58	46
Female	74	50	54
Mean age, years	6.5±2.2	6.6±2.8	6.8±2.0
BMI	16.8±3.4	17.4±2.6	17.2±3.0
SNOT-22	30.1±4.8	32.2±5.6	31.5±4.6
LKES	8.2±2.5	7.6±3.1	8.3±1.9
FVC, l	1.52±2.1	1.47±2.6	1.49±2.4
PEF, %	68.4±7.8	66.5±9.5	67.2±8.6
FEV, %	65.2±6.2	64.7±7.6	66.2±8.1

BMI, body mass index; SNOT-22, 22-item sinonasal outcome test; LKES, Lund-Kennedy endoscopy scores; FVC, forced vital capacity; PEF, peak expiratory flow; FEV, forced expiratory volume; rhLPHN3 Ab, recombinant human latrophilin 3 antibody.

USA) was used for analyzing NF- κ B activation using an assay of the photons produced (measured in relative light units).

DNA binding activity. Airway smooth muscle cells were isolated from pediatric asthma patients on week 0 and 16. DNA binding activity was determined by DNA binding and antitrypanosomal activity as reported previously (21).

Outcome measurement. SNOT-22 and LKES tests were used for assessing the efficacy of salbutamol, montelukast and rhLPHN3 Ab treatments in children with pediatric asthma at the baseline and post-treatment at week 16. Clinical pediatric asthma scores were evaluated as described in previous studies (22,23).

Lung function testing. Lung function tests, including the FEV percentage, FVC and PEF, were conducted using a Jaeger MasterScreen Pulmonary Function Testing system (Jaeger; BD Biosciences, Franklin Lakes, NJ, USA) to evaluate the tidal breathing flow volume at the baseline and post-treatment. The procedures were performed according to the manufacturer's instructions.

Efficacy and safety assessments. Subjective and objective outcome measurements were used to assess the efficacy of salbutamol, montelukast and rhLPHN3 Ab on children patients with pediatric asthma. In order to minimize bias, a single physician measured the scores at the preoperative baseline and post-treatment. Efficacy assessments, including the median percent reduction scores and response rate, were analyzed in children with pediatric asthma from baseline during the 16-week and double-blind period in the presence of rhLPHN3 Ab (12 mg/day). The safety assessments of the most frequent treatment-emergent adverse events were

evaluated in all children with pediatric asthma who received the study drug.

ELISA for detection of plasma concentration. The levels of interleukin (IL)-10 (BMS614-2), IL-17 (39-8170-65), IL-4 (39-8041-65), MMP-9 (BMS2016-2), interferon- γ (IFN- γ ; BMS216TEN) and TGF- β (BMS249-4TEN) in the serum of children patients with asthma were investigated using commercial ELISA kits (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. Absorption was measured at 450 nm with an ELISA reader, and was subsequently converted to the concentrations of IL-10, IL-17, IL-4, MMP-9, IFN- γ and TGF- β (24).

Evaluation of toxicity. The median overall duration of treatment for dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) was 16 weeks for rhLPHN3 Ab dosing cohorts (1, 6, 12, 24 and 30 mg) and each group had 20 patients. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria (version 3.0). DLT and MTD of rhLPHN3 Ab were evaluated by hypertension and proteinuria grade, as described previously (25).

Statistical analysis. All data are reported as the mean \pm standard error of the mean. Differences between mean values were assessed by Student's t-test for unpaired data. Comparisons of data between multiple groups were performed with analysis of variance. Responder rates and treatment-emergent adverse events were analyzed by χ^2 test. $P < 0.05$ was considered as an indicator of statistically significant differences.

Results

Characteristics of patients with pediatric asthma. In total, 342 patients with pediatric asthma were recruited into the present analysis. The characteristics of the children patients are summarized in Table I. Out of 342 patients, 134 received salbutamol, 108 received montelukast and 100 received rhLPHN3 Ab treatment. The number of male and female patients was approximately equal. In addition, the scores of asthma in children were analyzed by SNOT-22 and LKES determination in order to evaluate the extent of asthma at the baseline and following salbutamol, montelukast and rhLPHN3 Ab treatment. The patients did not receive any other medications during the treatment period.

Duration of treatment, DLT and MTD of rhLPHN3 Ab. The overall duration of salbutamol, montelukast and rhLPHN3 Ab treatments were identified as 16 weeks for all treatments. The various doses of rhLPHN3 Ab were 1, 6, 12, 24 and 30 mg, which were used to evaluate the optimal dosage. Data presented in Table II revealed that the DLT and MTD of rhLPHN3 Ab were 24 and 30 mg, respectively. Furthermore, analysis indicated that the common treatment-emergent adverse events of rhLPHN3 Ab treatment were hypertension, proteinuria, fatigue, hypertriglyceridemia, constipation and edema peripheral (Table II). Notably, the majority of patients with pediatric asthma required reduction of the drug dose due to cumulative toxicity following treatment with the MTD dose of rhLPHN3 Ab. Therefore, pediatric asthma patients

Table II. Treatment-emergence adverse events of rhLPHN3 Ab after 16 weeks treatment.

Adverse event	Total (n=100)	Dose of rhLPHN3 Ab		
		1-6 mg/day (n=40)	12-24 mg/day (n=40)	30 mg/day (n=20)
Hypertension	8	2	3	3
Proteinuria	5	1	2	2
Fatigue	3	0	1	2
Hypertriglyceridemia	3	1	1	1
Constipation	4	1	1	2
Edema peripheral	4	1	1	2

Treatment-emergent adverse events were analyzed by χ^2 test. rhLPHN3 Ab, recombinant human latrophilin 3 antibody.

Table III. Hypertension and proteinuria grades after 16 weeks treatment with rhLPHN3 Ab.

Adverse event	Total (n=37)	Dose of rhLPHN3 Ab		
		1-10 mg/day (n=12)	15 mg/day (n=15)	20 mg/day (n=10)
Hypertension	8	2	3	3
Grade 1	4	1	2	1
Grade 2	2	0	1	1
Grade 3	2	1	0	1
Proteinuria	5	1	2	2
Grade 1	2	0	1	1
Grade 2	2	1	1	0
Grade 3	1	0	0	1

Treatment-emergent adverse events were analyzed by χ^2 test. rhLPHN3 Ab, recombinant human latrophilin 3 antibody.

included in the present study received a dose of 12 mg/day rhLPHN3 Ab. Following administration of the last dose of rhLPHN3 Ab (12 mg), it was observed that the most common treatment-emergent adverse events were hypertension and proteinuria ($\geq 10\%$ each; Table III). Among the total cohort of patients, all pediatric asthma patients completed the overall maintenance period of the phase I study (n=100).

Efficacy of rhLPHN3 Ab treatment in patients with pediatric asthma. In order to investigate the efficacy of rhLPHN3 Ab treatment on the improvement of pediatric asthma, the therapeutic effects in 100 patients using the SNOT-22 and LKES tests. The onset frequency, duration of capillary bronchitis and asthma were compared among patients receiving treatments of salbutamol, montelukast and rhLPHN3 Ab. As shown in Fig. 1A, the present study data demonstrated that the onset frequency was significantly decreased in the three groups, while the outcomes in the rhLPHN3 Ab group were better as compared with the salbutamol and montelukast groups ($P < 0.01$). The results shown in Fig. 1B revealed that the duration of capillary bronchitis was relieved and 63 patients completely recovered subsequent to rhLPHN3 Ab treatment. Meanwhile, the duration

of asthma was decreased following treatment with salbutamol, montelukast and rhLPHN3 Ab (Fig. 1C). It was also observed that the number of days patients were awakened during the night was evidently decreased subsequent to 16-week treatment in the three groups (Fig. 1D). These data suggest that rhLPHN3 Ab is more efficient compared with salbutamol and montelukast for the treatment of pediatric asthma.

Analysis of inflammatory factors in pediatric asthma patients following treatment with rhLPHN3 Ab. The levels of various inflammatory factors (IL-10, IL-17, IL-4, MMP-9, IFN- γ and TGF- β) in the patients with pediatric asthma were analyzed in the present clinical study. As shown in Fig. 2A, the results demonstrated that IL-10 concentration was upregulated following treatment with salbutamol, montelukast and rhLPHN3 Ab. By contrast, the plasma concentration of IL-17 was decreased in all treatment groups, while IL-17 levels were significantly lower in the rhLPHN3 Ab group compared with salbutamol and montelukast groups ($P < 0.01$, Fig. 2B). In addition, rhLPHN3 Ab treatment downregulated MMP-9 plasma levels, which may contribute to inhibition of the inflammatory response (Fig. 2C). It was also observed

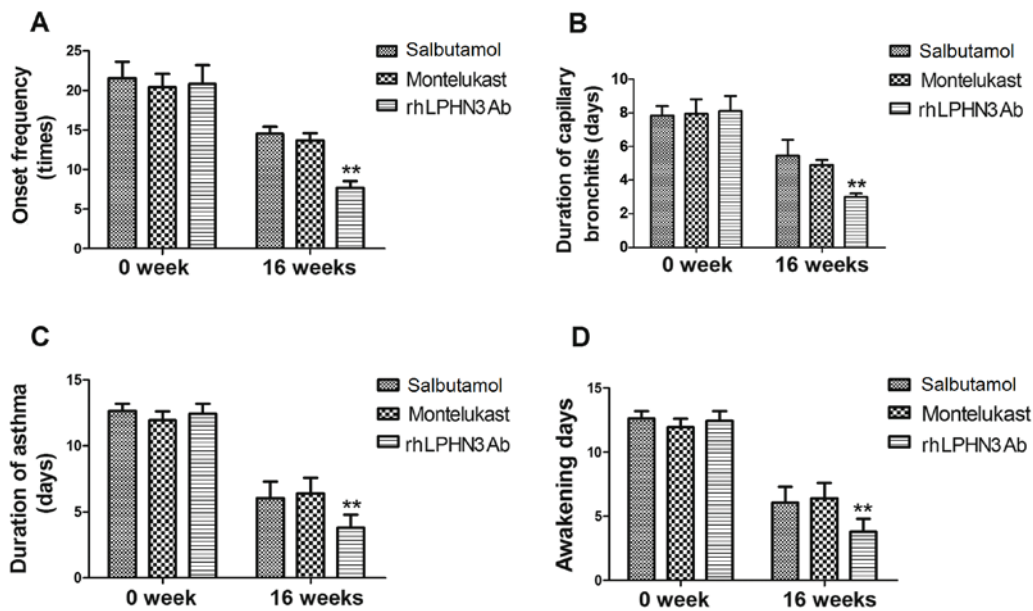


Figure 1. Efficacy of rhLPHN3 Ab treatment in patients with pediatric asthma. The (A) onset frequency, (B) duration of capillary bronchitis, (C) duration of asthma, and (D) number of the days patients were awakened during the night were evaluated prior to treatment (0 weeks) and following treatment (16 weeks). **P<0.01 vs. salbutamol and montelukast. rhLPHN3 Ab, recombinant human latrophilin 3 antibody.

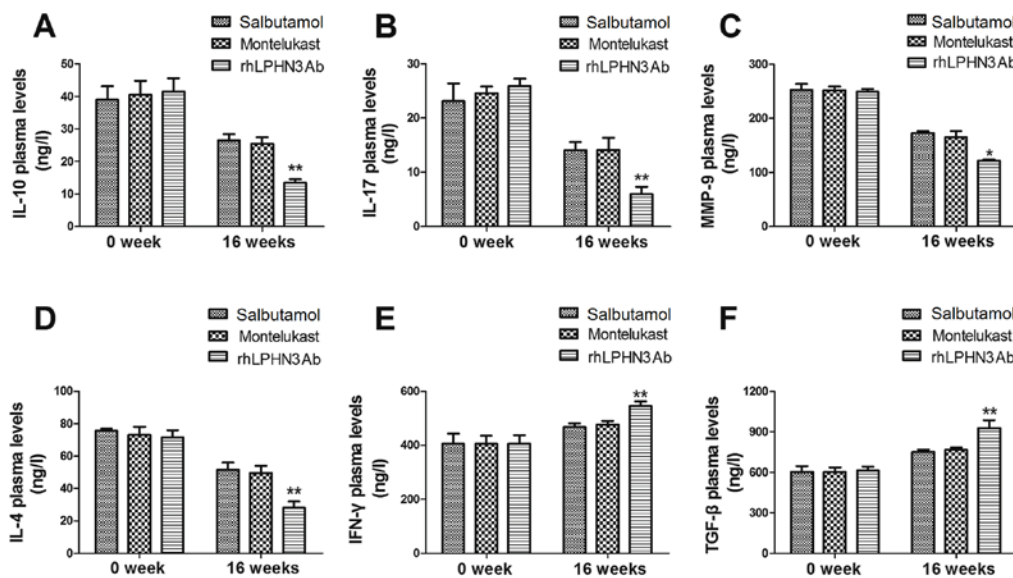


Figure 2. Analysis of alterations in inflammatory factor levels in patients with pediatric asthma following treatment with salbutamol, montelukast and rhLPHN3 Ab. (A) IL-10, (B) IL-17 and (C) MMP-9 plasma concentration levels were downregulated after treatment (16 weeks). (D) IL-4, (E) IFN-γ and (F) TGF-β plasma concentration levels were upregulated after treatment with salbutamol, montelukast and rhLPHN3 Ab. *P<0.05 and **P<0.01 vs. salbutamol and montelukast. rhLPHN3 Ab, recombinant human latrophilin 3 antibody. IL, interleukin; MMP-9, matrix metalloproteinase-9; IFN-γ, interferon-γ; TGF-β, transforming growth factor-β.

that IL-4 plasma concentration levels were significantly downregulated in rhLPHN3 Ab group compared with the salbutamol and montelukast groups (Fig. 2D). Furthermore, it was observed that IFN-γ and TGF-β concentration levels were higher in rhLPHN3 Ab group compared with the salbutamol and montelukast groups (Fig. 2E and F). These observations indicated that the inflammatory factor levels in the children were improved following treatment with rhLPHN3 Ab.

Analysis of potential mechanism mediated by rhLPHN3 Ab in airway smooth muscle cells. Previous studies have proposed

numerous signaling pathways that participate in the development and progression of pediatric asthma (26,27). In the present study, the rhLPHN3 Ab-mediated signaling pathway in airway smooth muscle cells was investigated. Initially, the E-selectin and MUC5AC expression levels in these cells were investigated. As shown in Fig. 3A-B, rhLPHN3 Ab treatment significantly decreased E-selectin and MUC5AC expression levels in the airway smooth muscle cells as compared with the salbutamol and montelukast groups. In addition, data revealed that rhLPHN3 Ab treatment markedly reduced the phosphorylation of PKA in the airway smooth muscle

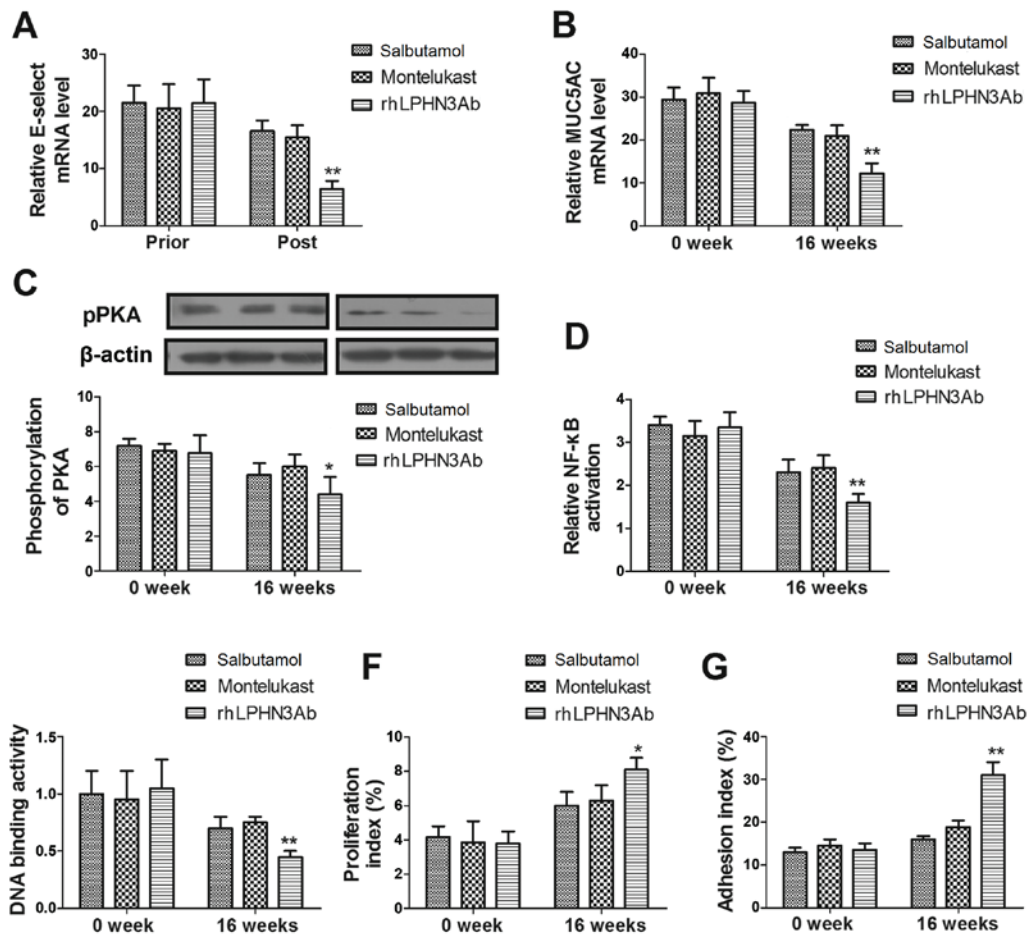


Figure 3. rhLPHN3 Ab treatment had a beneficial effect in pediatric asthma via NF- κ B signaling pathway regulation. Relative mRNA expression levels of (A) E-selectin and (B) MUC5AC in the airway smooth muscle cells was determined by polymerase chain reaction. (C) Phosphorylation of PKA in the airway smooth muscle cells determined by western blot analysis. Analysis of the (D) NF- κ B activation and (E) DNA binding activity, as well as alterations in the (F) proliferation and (G) adhesion of airway smooth muscle cells prior to and following treatment. * $P < 0.05$ and ** $P < 0.01$ vs. salbutamol and montelukast rhLPHN3 Ab, recombinant human latrophilin 3 antibody; MUC5AC, mucin 5AC; PKA, protein kinase A; NF, nuclear factor;

cells compared with the other treatment agents (Fig. 3C). Furthermore, NF- κ B activation and DNA binding activity were decreased in the cells following rhLPHN3 Ab treatment (Fig. 3D and E). rhLPHN3Ab also markedly improved the adhesion and proliferation of airway smooth muscle cells obtained from patients with pediatric asthma (Fig. 3F and G). These observations suggest that rhLPHN3 Ab treatment may improve pediatric asthma via the PKA-induced NF- κ B signaling pathway.

rhLPHN3Ab treatment improves the function of airway smooth muscle cells and lung contraction. The current study further analyzed the morphologic changes induced in airway smooth muscle cells after a 16-week treatment. As shown in Fig. 4A, rhLPHN3 Ab markedly inhibited airway mucus secretion when compared with that in the salbutamol and montelukast groups. It was also observed that the lung function of patients was improved following treatment with rhLPHN3 Ab, as determined by SNOT-22 and LKES testing (Fig. 4B and C). The results further revealed that rhLPHN3 Ab treatment markedly improved the contraction of the airways compared with the salbutamol and montelukast groups, as demonstrated by the FVC, PEF and FEV values (Fig. 4D-F). These results suggest that rhLPHN3 Ab contributes to the

improvement of pediatric asthma by regulating the activity of the airway smooth muscle cells.

Discussion

According to previous studies, immune dysfunction and abnormal expression of inflammation factors are closely associated with capillary bronchial asthma, bronchitis and other infant asthmatic diseases (28-30). Notably, Li *et al.* (31) have demonstrated that the PKA-dependent NF- κ B signaling pathway is a novel target for drug action in asthma therapy. In addition, evidence indicated that latrophilin receptors are associated with heterogeneous behavioral disorder (32), which is also a novel target in asthma (14,33). In the present study, the changes in inflammation factor levels and the mechanism underlying the rhLPHN3 Ab-mediated signaling pathway were analyzed in patients with pediatric asthma. The clinical outcomes revealed that rhLPHN3 Ab treatment decreased the levels of plasma pro-inflammatory factors, pulmonary pathological alterations, MUC5AC and E-selectin expression levels and mucus hyper-secretion in patients with pediatric asthma. In addition, data in the present analysis also revealed that rhLPHN3 Ab significantly facilitated the proliferation and adhesion of airway smooth

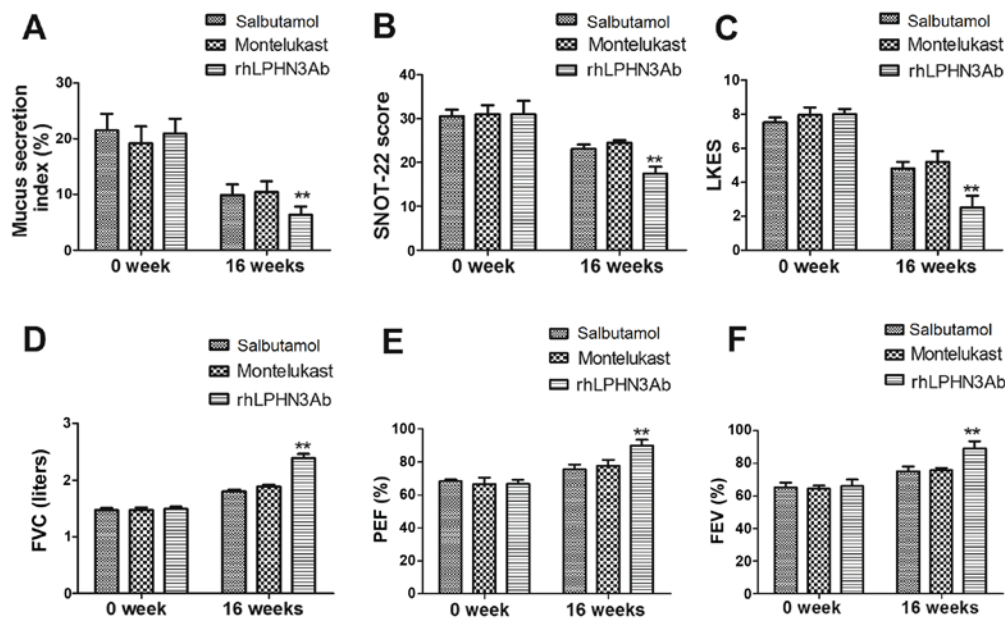


Figure 4. Analysis of functional recovery of lungs subsequent to rhLPHN3 Ab treatment. (A) Alterations in airway mucus secretion in patients with pediatric asthma are shown. Lung function after the treatment with rhLPHN3 Ab was determined by (B) SNOT-22 and (C) LKES examination. Contraction in the airway smooth muscles was analyzed by (D) FVC, (E) PEF and (F) FEV in patients with pediatric asthma following rhLPHN3 Ab. ** $P < 0.01$ vs. salbutamol and montelukast. rhLPHN3 Ab, recombinant human latrophilin 3 antibody; SNOT-22, 22-item sinonasal outcome test; LKES, Lund-Kennedy endoscopy scores; FVC, forced vital capacity; PEF, peak expiratory flow; FEV, forced expiratory volume.

muscle cells in patients with pediatric asthma after 16-week treatment. Furthermore, it has been suggested that rhLPHN3 Ab decreased NF- κ B levels and improved the contraction of the airways through regulation of the PKA-mediated NF- κ B signaling pathway. These findings indicate that rhLPHN3 Ab may be an efficient agent for the treatment of pediatric asthma.

Currently, pediatric asthma presents the characteristics of chronic inflammatory responses and systolic dysfunction of the airway, in which marked changes in airway smooth muscle cell contraction are observed. In a recent study, a systematically review revealed the associations between inflammatory responses and asthma severity in pediatric asthma, which indicated that IFN- γ , IL-10 and IL-17 may serve as prognostic indicators of pediatric asthma (34). In addition, various strategies targeting inflammatory responses to improve airway remodeling in asthma physiopathology have been investigated, and the results of these studies support the hypothesis that modulating the allergic inflammation improved asthma physiopathology in patients with asthma in animals model and clinical trials (35-38). Furthermore, previous reports have indicated that the pathogenesis of airway inflammation in asthma patients may be associated with the normal function and maintenance of the airway smooth muscle cells (39-41). To investigate the therapeutic effects of rhLPHN3 Ab in the treatment of pediatric asthma, the current study analyzed the cytokine expression levels in the plasma of patients. It was observed that rhLPHN3 Ab significantly inhibited IL-17, MMP-9 and IL-10 levels, while it enhanced IL-4, IFN- γ and TGF- β expression levels in the peripheral blood. This inhibitory effect may contribute to the morphological changes of airway smooth muscle cells. It was indicated that 16-week rhLPHN3 Ab treatment improved BMI, SNOT-22, LKES, FVC, PEF and FEV.

Clinical manifestations of asthma include the first onset of capillary bronchitis, typical bronchial asthma, and chronic inflammation of the airway, which have been regarded as evaluation criteria for patients with asthma (42-44). In addition, studies have indicated that the number of times patients were awakened by asthma during the night is also an important factor that evaluates the efficacy of the childhood asthma treatment (45,46). Furthermore, contraction of the airways is another factor to evaluate the extent of capillary bronchitis, which may be an early signal of bronchial asthma in children (47,48). In the current study, the efficacy of rhLPHN3 Ab on the onset frequency, duration of capillary bronchitis, asthma and times awakened was investigated and compared with the effects of salbutamol and montelukast serving as the controls. The findings revealed that rhLPHN3 Ab evidently improved the clinical manifestations in patients with pediatric asthma.

Notably, it has been reported that PKA-mediated NF- κ B signaling pathway may be associated with the progression of pediatric asthma (49,50). In addition, previous studies demonstrated that liraglutide possesses an anti-inflammatory potential through inactivation of TNF- α expression and activation of NF- κ B signaling pathway in the progression of pediatric asthma (51,52). Furthermore, a previous report indicated that inhibition of NF- κ B signaling pathway led to the amelioration of inflammation and airway hyperresponsiveness in a mouse asthma model (53). Similarly, the current study observed that rhLPHN3 Ab inhibited the inflammatory responses through PKA-mediated NF- κ B signaling pathway, which may contribute to the recovery of patients with pediatric asthma.

In conclusion, the clinical outcomes of patients included in the present study suggested that clinical treatment with rhLPHN3 Ab exerted various beneficial effects on the secretion

of inflammatory mediators in pediatric asthma, which contributes to the recovery of typical bronchial asthma, and chronic inflammation of the airway in patients with pediatric asthma. These findings contribute to better understand the immunologic mechanism underlying the rhLPHN3 Ab-mediated treatment for pediatric asthma.

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