



Desmoglein 3 gene mediates epidermal growth factor/epidermal growth factor receptor signaling pathway involved in inflammatory response and immune function of anaphylactic rhinitis



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ABSTRACT

Objective: To investigate the effects of desmoglein 3 (DSG3) gene mediating epidermal growth factor/epidermal growth factor receptor (EGF/EGFR) signaling pathway on inflammatory response and immune function of anaphylactic rhinitis (AR).

Methods: Ten of the seventy male BALB/c mice were randomly selected as the normal control group, and the remaining 60 were used to construct the AR mice model. AR model mice were divided into 6 groups: model group (instilled with 5 μ L saline), empty vector group (instilled with 5 μ L of liposome and empty vector mixture), siRNA-DSG3 group (instilled with 5 μ L of liposome and siRNA-DSG3 carrier mixture), AG1478 group (instilled with 5 μ L of EGF/EGFR inhibitor AG1478), siRNA-DSG3 + AG1478 group (instilled with 5 μ L of liposome and siRNA-DSG3 carrier and EGF/EGFR inhibitor AG1478 mixture) and oe-DSG3 group, 10 in each group. After taking serum, each group of mice was sacrificed to get nasal mucosa tissues. HE staining was used to observe the pathological changes of nasal mucosa tissues in each group. The expression levels of DSG3, EGF and EGFR in nasal mucosa tissues of mice in each group were detected by qRT-PCR and western blot methods respectively. TUNEL staining was used to observe the apoptosis of nasal mucosa cells in mice. The expression of IgE, INF- γ , TNF- α , IL-2, IL-4 and IL-6 in serum of mice was determined by ELISA method. The immune adhesion function of red blood cells was detected by complement sensitization yeast hemagglutination method.

Results: All the mice with AR showed different degrees of nasal mucosa injury and inflammatory cell infiltration, and silencing DSG3 or inhibiting the activity of EGF signaling pathway could alleviate the nasal mucosa injury. Compared with control group, the INF- γ and IL-2 levels of serum in AR model mice were significantly decreased; IgE, TNF- α , IL-4 and IL-6 levels were significantly increased (all $P < 0.05$); the mRNA expression levels and protein levels of DSG3, EGF and EGFR were significantly increased (all $P < 0.05$); C₃b receptor rosette rate and Ic rosette rate were significantly decreased (all $P < 0.05$). Detected by ELISA method, the expression levels of IgE, TNF- α , IL-4 and IL-6 were increased, while the expression levels of INF- γ and IL-2 were decreased after DSG3 silencing or using AG1478. Detected by qRT-PCR and western blot methods, the expression of DSG3, EGF and EGFR did decrease after DSG3 silencing. There was no significant difference in the EGF and EGFR expression between DSG3 silencing and using AG1478, and the expression decreased even more under the double effect. The mRNA and protein expression levels of DSG3, EGF and EGFR in the nasal mucosa tissues of mice with

Abbreviations: DSG3, desmoglein 3; EGF/EGFR, epidermal growth factor/epidermal growth factor receptor; AR, anaphylactic rhinitis; AI, apoptotic index

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overexpression of DSG3 plasmid were significantly higher than those of normal mice (all $P < 0.05$).

Conclusion: Silencing DSG3 gene can inhibit the activation of EGF signaling pathway, alleviate the inflammation of AR nasal mucosa, and enhance red blood cells immune adherence function.

1. Introduction

Anaphylactic rhinitis (AR) is also known as allergic rhinitis, mainly refers to non-infectious inflammatory diseases of the nasal mucosa caused by individuals after exposure to atopic allergens [1]. Atopic individuals (allergic constitution), specific antigen leading to the body's immune response and the combination of atopic individuals and specific antigen are the three important causes of AR; it can be seen that both external and internal immune factors are important causes of AR pathogenesis [2,3]. The current clinical drug therapy for AR mainly focuses on regulating the imbalance of the immune function in patients [4,5].

Desmoglein 3 (DSG3) is one of the specific antigens of autoimmune diseases - pemphigus vulgaris, and its expression product DSG3 protein is an important component of the desmosome transmembrane [6]. Studies have confirmed that specific antigen DSG3 can produce auto-reactive T cell activation under certain stimulation conditions, which leads to the imbalance of Th1/Th2 and Tc1/Tc2 cell differentiation and promotes the pathogenesis of pemphigus vulgaris [7–9]. However, whether it is related to the pathogenesis of AR in autoimmune diseases is not clear. Epidermal growth factor receptor (EGFR), also known as c-erbB1, a study has been confirmed that EGFR signaling pathway can be involved in a variety of biological behaviors such as cell proliferation and movement [10]. In recent years, study has confirmed that EGFR plays an important role in the chronic inflammatory response of the lower airway, and is an important factor in the repair and reconstruction of bronchial outcomes in patients with chronic bronchial asthma [11]. Studies have confirmed that EGFR is highly expressed in both types of sinusitis tissues [12,13]. In addition, a study has confirmed that EGFR signaling pathway is significantly activated in chronic sinusitis and allergic rhinitis, suggesting that it is involved in the pathogenesis of rhinitis, and the regulation of EGFR signaling pathway is very important for the treatment of this disease [14]. A study on the pathogenesis of systemic pemphigus have found that DSG3 gene can influence the progression of systemic pemphigus by mediating the EGFR signaling pathway [15]. However, it is unclear whether the DSG3 gene and the EGFR signaling pathway affect the onset of AR. AG1478, as a small molecular weight EGFR inhibitor, has high selectivity for EGFR. It can inhibit the phosphorylation of EGFR and block the activity of EGFR signaling pathway through competitive inhibition of intracellular catalytic sites of EGFR and ATP binding [16]. In this study, we established AR model mice, silenced DSG3 in their tissues or using AG1478 to investigate the association between DSG3 gene and EGF/EGFR signaling pathway and its effects on inflammatory response and immune function of AR, hoping to provide new targets for molecular targeted therapy of AR.

2. Methods

2.1. Construction of AR mice model

Seventy male SPF BALB/c mice weighing 18–20 g were selected (purchased from the Laboratory Animal Center of Southern Medical University). Ten of them were randomly selected as the normal control group, and the remaining 60 were used to construct the AR mice model. All mice were modeled one week later after adapting to the environment. Firstly, OVA:Al(OH)₃ and normal saline were prepared at a ratio of 1:5, and the concentration of OVA was 1 mg/mL, which was dripped into the bilateral nostrils of the mice, 20 μ L each time, twice a day, and continuously dripped for 10 days. Fifty AR mice were intranasally

instilled with 5 mg/mL Al(OH)₃ solution. After 5 days' interval, they were stimulated with 5% OVA solution. Each side of the nostril was 20 μ L, and it was stimulated once every 3 days, continuously stimulated for 11 times. The control group was replaced with saline. After modeling, the score criteria were set according to the mouse nasal itching, sneezing times and the severity of rhinorrhea, the length of time to appear, and the scores were recorded. The observation time was set at 30 min for each time, and the mice model was successfully constructed if 3 symptom scores overlap 5 points or more [17]. Score criteria: nasal itching: accidental scratching of the nose of a single forelimb was 1 point, both forelimbs scratched the nose was 2 points, keep wiping nose everywhere was 3 points; sneezing: 1 to 3 was 1 point, 4 to 10 was 2 points, more than 10 was 3 points; rhinorrhea: Flowing to the anterior naris was 1 point, flowing over the anterior naris was 2 points, and flowing to the face everywhere was 3 points [18]. The total score was recorded by the superposition method. The mice with successful modeling were selected for the follow-up study. The animal experiment was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University. The experimental process was in accordance with the principles of protection and use of experimental animals.

2.2. Preparation of liposome and plasmid mixture

The 2 μ L of Lipofectamine 2000 liposomes (Thermo Fisher, USA) was consecutively incubated in 48 μ L OptiMEM medium (Shanghai Yezhou Biotechnology Co., Ltd., China) for 5 min. The specific procedure was carried out according to Lipofectamine 2000 liposomes (Thermo Fisher, USA) instructions. Then the incubated solution was mixed with 20 nmol siRNA-DSG3 plasmid (Shanghai Jima Company, China), oe-DSG3 plasmid and empty vector plasmid (Shanghai Jima Company, China). The mixture was diluted by adding them into 50 μ L OptiMEM medium (Shanghai Guduo Biotechnology Co., Ltd., China) and then placed at room temperature for 20 min. The state of plasmid and liposome mixture was unstable, so it should be prepared before each nasal administration of mice.

2.3. Grouping of AR mice

The 60 mice that were successfully modeled were selected to administrate on the first day and the second day with a P20 hand pipette respectively to drip into the nasal cavity with a mixture of liposome and each plasmid or an EGF signaling pathway inhibitor, and they were divided into 6 groups: model group (instilled with 5 μ L saline), empty vector group (instilled with 5 μ L of liposome and empty vector mixture), siRNA-DSG3 group (instilled with 5 μ L of liposome and siRNA-DSG3 carrier mixture), AG1478 group (instilled with EGF/EGFR inhibitor AG1478, 100 ng/mL, Sigma Company, USA), siRNA-DSG3 + AG1478 group (instilled with 5 μ L of liposome with siRNA-DSG3 carrier and 5 μ L of EGF/EGFR inhibitor AG1478 mixture) and oe-DSG3 group, 10 in each group.

2.4. Tissues sampling and trimming

The mice in each group were anesthetized and sacrificed by venous bloodletting 48 h after administration. The mice were fasted for 12 h before bloodletting and their venous blood were collected. After successful blood collection, heparin (Shandong Chenzhong Biopharmaceutical Co., Ltd., China) was added to part of the blood for anticoagulant reserve. The other part was placed at 4 °C, after standing for 3 min, it was centrifuged at 3000 r/min for 15 min. The supernatant

was taken and stored at -80°C , for the determination of the expression of inflammatory factors by ELISA method. The mice were placed on an ultra-clean table ice tray and the jaws of the mice were freed. The nasal septum was exposed after cutting the nasal cavity along the middle line. The bilateral mucosa of the nasal septum was stripped off. The nasal septum was washed with 0.2% DEPC saline and fixed in 10% neutral formaldehyde solution for 24 h. After the fixation was completed, it was placed in a dark environment for use.

2.5. HE staining

The nasal mucosa specimens fixed with formaldehyde were serially sliced $4\mu\text{m}$ after paraffin embedding, and stained with Hematoxylin-Eosin (HE) staining. The operation steps are: xylene dewaxing for 2 times, 15 min each time; anhydrous ethanol dehydrating for 2 times, 5 min each time; 90% ethanol dehydrating for 5 min; 80% ethanol dehydrating for 5 min; washing with water for 5 min. Hematoxylin staining: hematoxylin solution was stained for 5 min, washed with water until it became blue; 1% hydrochloric acid ethanol was rapidly differentiated, washed with water until it turned blue, then dehydrated, transparentized, and sealed. 80% ethanol dehydrating for 5 min; 90% ethanol dehydrating for 5 min; absolute ethanol dehydrating for 2 times, 5 min each time; xylene transparentizing for 2 times, 15 min each time; neutral gum sealed. Part of them was used for HE staining light microscope histopathological examination and were photographed (H-7500, Hitachi Company, Tokyo, Japan), and the color development of each group was observed. On the Image-Pro Premier image analysis system, different groups were taken at 200 times magnification and images were collected randomly. The other part was counted under the HE staining light microscope to calculate the number of eosinophils in any 15 fields.

2.6. qRT-PCR

Total RNA was extracted from fresh nasal mucosa tissues of mice by ultrapure RNA extraction kit (QIAGEN Company, Germany). DSG3, EGF and EGFR primers were designed and synthesized by Oke Biotech Co., Ltd. (China). See Table 1. A total of $20\mu\text{L}$ of the reverse transcription system was carried out according to the instructions of TaqMan MicroRNA Assays Reverse Transcription Primer (batch No. 4366596, thermo scientific, Waltham, MA, USA), and the reaction conditions were set at 42°C , 30–50 minutes (reverse transcription reaction); 85°C , 5 s (reverse transcriptase inactivation reaction). Fluorescence quantitative PCR was performed according to the instructions of the SYBR® Premix Ex Taq™ II Kit (RR820A, Xingzhi Biotechnology Co., Ltd.). Reaction system $50\mu\text{L}$: SYBR® Premix Ex Taq™ II ($2\times$) was $25\mu\text{L}$, PCR upstream primer was $2\mu\text{L}$, PCR downstream primer was $2\mu\text{L}$, ROX Reference Dye ($50\times$) was $1\mu\text{L}$, DNA template was $4\mu\text{L}$, and ddH_2O was $16\mu\text{L}$. Fluorescence quantitative PCR was performed under ABI PRISM® 7300 (type of Prism® 7300, Shanghai Kunke Instrument and Equipment Co., Ltd., Shanghai, China) system. The reaction conditions were: pre-denaturation at 94°C for 10 min, denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and then extension at 72°C for 1 min after 40 cycles. GAPDH was regarded as an internal reference for DSG3, EGF, EGFR, p38MAPK and Src (abs830032, Ai Bixin Biotechnology Co., Ltd., China).

2.7. Western blot

The nasal mucosa cells of each group were taken, dissolved and washed 3 times with cold PBS (Biyuntian, Jiangsu, China). Whole protein lysate was added and then lysed on ice for 10 min to get the protein. After protein extraction, the protein was quantified by BCA method, and SDS-PAGE was performed in the 100 V lane. After electrophoresis, the protein was transferred to the NC membrane at 30 mA and 120 min. Block solution (5% BSA/TBST) was used to block the

membrane for 60 min. After washing the membrane, rabbit anti-mouse DSG3 polyclonal IgG antibodies ($1:1000$, ab203692, Abcam, UK), rabbit anti-mouse EGF polyclonal IgG antibodies ($0.1\mu\text{g/mL}$, ab9695, Abcam, UK), rabbit anti-mouse EGFR polyclonal IgG antibodies ($1:1000$, ab32077, Abcam, UK), rabbit anti-mouse p38MAPK polyclonal IgG antibodies, rabbit anti-mouse Src polyclonal IgG antibodies, rabbit anti-mouse Fyn polyclonal IgG antibodies, and rabbit anti-mouse GAPDH polyclonal IgG antibodies ($1:2500$, ab9485, Abcam, UK) were added and they were stayed overnight at 4°C . After washing the membrane again, HRP-labeled sheep anti-rabbit IgG ($1:2000$, ab6721, Abcam, UK) was added and incubated at 37°C for 4 h. The TBST solution on the membrane was sucked out with a water-absorbent filter paper and placed on a clean glass plate. The ECL fluorescent detection kit (BB-3501, Ameshame Company, UK) was taken and the same amount of liquid A and liquid B were mixed and then added to the membrane and put into the gel imager for exposure imaging. Photographed by Bio-Rad Image Analysis System (BIO-RAD Company, USA), analyzed by Quantity One v4.6.2 software, and the relative protein content was expressed by the gray value of the corresponding protein band/the gray value of the GAPDH protein band. The experiment in each group was repeated 3 times.

2.8. TUNEL staining

Five pieces slice after dewaxing into the water mentioned before were took, $50\mu\text{L}$ of 1% dilution of proteinase K was added to each piece, and incubated them in a 37°C incubator for 30 min, rinsing with PBS for 3 times, 5 min each time. The endogenous POD activity was eliminated by adding methanol solution containing 0.3% H_2O_2 , incubated at 37°C for 30 min at room temperature, and washed with PBS for 3 times, 5 min each time. TUNEL reaction solution (Roche Company, Switzerland) was added, incubated in a wet box at 37°C for 1 h in the dark, and rinsed 3 times with PBS for 5 min each time. A total of $50\mu\text{L}$ of Converter-POD (Roche Company, Switzerland) was added, incubated in a wet box at 37°C for 1 h, and rinsed 3 times with PBS for 5 min each time. A total of 2% DAB color-substrate solution was added and incubated for 15 min at room temperature. The reaction was terminated by adding distilled water after brown yellow nuclei in cells were observed under microscope. After the reaction was terminated, rinsed with PBS for 3 times, 5 min each time. Hematoxylin counterstained, distilled water to terminate the reaction, 50%, 70%, 90%, 100% gradient ethanol dehydration, sealed with neutral gum after xylene transparent. Observed under an optical microscope, each slice randomly took 10 fields of view, positive cells and nasal mucosa cells were counted respectively. The cells with brown yellow color were apoptotic positive cells, while the cells with blue color were normal cells. Taking

Table 1
qRT-PCR primer sequences.

Items	Sequences
DSG3	F: 5'-GGAGGAACAGACTAACAGGC-3' R: 5'-ACCATCAGGAGGGCCAGAGA-3'
EGF	F: 5'-GGTGGAAAGTTTGGATGTAAAGC-3' R: 5'-AGTGAAGACCCTGATAACCCAC-3'
EGFR	F: 5'-AGAAGATGGCATCCGCAAGT-3' R: 5'-ATTGGG TCTCCCGAAGAGTT-3'
p38MAPK	F: 5'-TCGAGACCGTTTCAGTCCATC-3' R: 5'-GGGTC ACCAGGTACACGTCATT-3'
Src	F: 5'-CGGGATCCCGATGGGCAG-CAACAAGAGC-3' R: 5'-CGGAATTCGCTATAGGT-TCTCCCGGGC-3'
Fyn	F: 5'-AAGGAGATTGGTGGGAAGCC-3' R: 5'-CTGCCTGGATAGAGTCAACTGG-3'
GAPDH	F: 5'-GGTGAAGGTCGGTGTGAACGGATTGG-3' R: 5'-TGTGCCGTGAATTGCGGTGAGTGG-3'

DSG3: desmoglein 3; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor.

the average value, the number of brown yellow cells/blue cells was taken as apoptotic index (AI) of nasal mucosa cells.

2.9. ELISA method

The ELISA kit (purchased from Wuhan Huamei Bioengineering Co., Ltd.) was taken out from the refrigerator 20 min in advance and unfrozen to room temperature. After the serum samples of each group were taken out, the antigen was serially diluted by means of carbonate coating buffer solution (pH 9.6). The dilution concentrations were: 180 pg/mL, 90 pg/mL, 45 pg/mL, 23 pg/mL, 11 pg/mL, and 6 pg/mL. 50 μ L of different concentrations of antigen were added to each standard hole. The blank hole and sample hole were set up. 50 μ L sample diluent was added into the blank hole, 40 μ L sample diluent and 10 μ L sample to be tested were added into the sample hole. Incubated it at 37 °C for 1 h, and then dried. 50 μ L of the enzyme standard reagent was added to each hole of the sample hole, and it was incubated at 37 °C for 1 h, washed with buffer solution for 5 times, and then dried. 100 μ L of the prepared TMB (batch EL0001, Huzhou Yingchuang Biotechnology Co., Ltd., Zhejiang, China) substrate solution was added to each reaction hole, and incubated at 37 °C for 15 min in the dark. A terminating solution of 50 μ L was added to terminate the color development, and the optical density (OD) value of each hole was measured at a wavelength of 450 nm by ELIASA (Simmerfly, USA). The concentration of the standard solution was taken as the abscissa and the OD value was taken as the ordinate. The regression equation of the standard curve was calculated in the computer. The OD value of the sample was substituted into the equation to calculate the sample concentration, that is, the actual concentration of the sample (The experiment was repeated three times here). After the transfected cells were cultured, the supernatant of the cells was collected, centrifuged at 1000 r/min for 10 min, and IgE, INF- γ , IL-1 β , IL-4, and IL-17 were detected by ELISA method. The experimental procedures were the same as above.

2.10. Complement sensitization yeast hemagglutination method

A total of 0.1 mL venous blood was taken from each group and mixed with a little heparin. Red blood cells were rinsed 3 times with normal saline and prepared into 1×10^8 /mL suspension. The suspension was placed in 2 tubes and 0.075 mL of red blood cells suspension was added to each tube. In the first tube, 0.075 mL complement sensitization yeast polysaccharide solution was added (Immune Department of Changhai Hospital, Second Military Medical University, Shanghai, China), and in the second tube, 0.075 mL non-sensitization yeast polysaccharide solution was added (Immune Department of Changhai Hospital, Second Military Medical University, Shanghai, China). After mixing the 2 tubes well, put them in 37 °C water bath for 30 min, then took out and added 0.1 mL normal saline, next added 0.05 mL 0.25% glutaraldehyde, mixed them well again, smeared horizontally, staining with Wright's stain, and conducted microscopic examinations. Two or

more yeasts in one red blood cell node were a garland, which counted for 200 red blood cells, the percentage was calculated. The first tube was the C₃b receptor rosette rate and the second tube was the Ic rosette rate.

2.11. Effect of DSG3 overexpression on EGFR pathway

The 10 male SPF BALB/c mice that were successfully modeled were selected to administrate on the first day and the second day with a P20 hand pipette to drip into the nasal cavity with a mixture of 5 μ L of liposome and oe-DSG3 (oe-DSG3 group).

The mice were anesthetized and sacrificed by venous bloodletting 48 h after administration. The mice were placed on an ultra-clean table ice tray and the jaws of the mice were freed. The nasal septum was exposed after cutting the nasal cavity along the middle line. The bilateral mucosa of the nasal septum was stripped off. The nasal septum was washed with 0.2% DEPC saline, then stored at -80°C, and was subsequently used for qRT-PCR and western blot detection.

2.12. Statistical analysis

All data were analyzed by SPSS21.0 (SPSS, Inc, Chicago, IL, USA) statistical software. The measurement data was expressed by mean $\pm$ standard deviation ($\bar{x} \pm s$). The comparison between multiple groups was analyzed by one-way ANOVA, and the SNK method was used for comparison between two groups. The enumeration data was expressed by number/percentage (n/%), and the comparison of multiple sample rates was used the chi-square test of line * list data, or Fisher's exact test. There is a significant difference at $P < 0.05$.

3. Results

3.1. General information of mice in each group

The nasal symptoms score of mice in each group after treatment was shown in Fig. 1. Compared with control group, the scores of nasal itching, sneezing and rhinorrhea in model group, empty vector group, siRNA-DSG3 group, AG1478 group and siRNA-DSG3 + AG1478 group were significantly increased (all $P < 0.05$). Compared with model group, the scores of nasal itching, sneezing and rhinorrhea in siRNA-DSG3 group, AG1478 group and siRNA-DSG3 + AG1478 group were significantly decreased (all $P < 0.05$). There was no significant difference between model group and empty vector group in nasal itching, sneezing and rhinorrhea scores (all $P > 0.05$). Compared with siRNA-DSG3 group, there was no significant difference in the scores of nasal itching, sneezing and rhinorrhea in AG1478 group ($P > 0.05$), while the siRNA-DSG3 + AG1478 group had a significant decrease in the scores of nasal itching, sneezing and rhinorrhea (both $P < 0.05$). The results showed that silencing DSG3 gene or inhibiting the activation of EGF signaling pathway could alleviate AR syndrome.

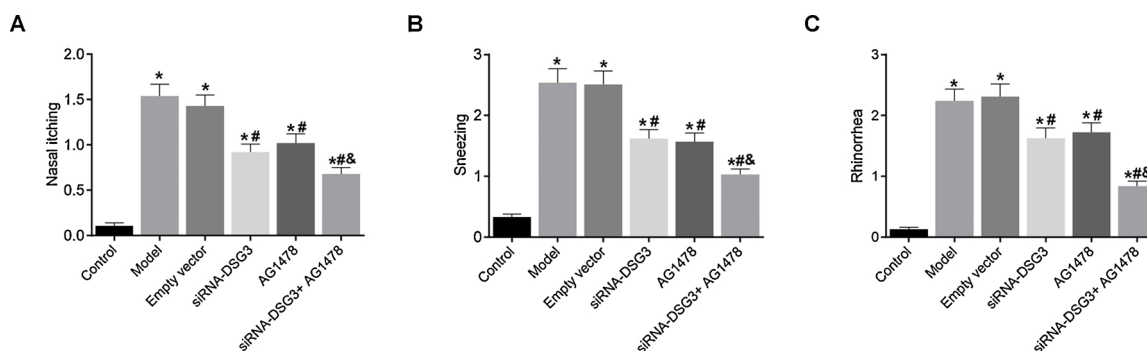


Fig. 1. Nasal symptoms score of mice in each group. A. Nasal itching score of mice in each group. B. Sneezing score of mice in each group. C. Rhinorrhea score of mice in each group. Compared with control group, * $P < 0.05$; compared with model group, # $P < 0.05$; compared with siRNA-DSG3 group, & $P < 0.05$.

3.2. Pathological changes of nasal mucosa tissues of mice in each group

The pathological changes of the nasal mucosa tissues of mice in each group were observed by HE staining. See Fig. 2. In the control group, the nasal mucosal epithelium structure was intact, arranged neatly, without thickening, and the proliferation of goblet cells was not obvious, and inflammatory cells were not common. The nasal mucosa structure of the model group and the empty vector group were loose and disordered, and the goblet cells were proliferated, showing chronic inflammation. This showed that the experiment was successful in modeling. The inflammatory reaction in the siRNA-DSG3 group, the AG1478 group, and the siRNA-DSG3 + AG1478 group was lighter than that in the model group.

3.3. mRNA and protein expression levels of EGF, EGFR, p38MAPK, Src and Fyn in nasal mucosa tissues of normal mice and AR model mice

qRT-PCR and western blot were used to detect the mRNA and protein expression levels of EGF, EGFR, p38MAPK, Src and Fyn in nasal mucosa tissues of control group and model group. The results showed that compared with control group, the mRNA and protein expression levels of EGF, EGFR, p38MAPK, Src and Fyn in nasal mucosa tissues of model group were significantly increased (both $P < 0.05$, Fig. 3). Among them, the EGF pathway has the largest changes in related factors, and the changes in p38MAPK, Src and Fyn are not as obvious as the EGFR pathway.

3.4. mRNA expression levels of DSG3, EGF and EGFR in nasal mucosa tissues of mice in each group

qRT-PCR was used to detect mRNA expression levels of DSG3, EGF and EGFR in nasal mucosa tissues of mice in each group. See Fig. 4. The results showed that compared with control group, the mRNA expression levels of DSG3, EGF and EGFR in nasal mucosa tissues of mice in model group, empty vector group, siRNA-DSG3 group, AG1478 group and siRNA-DSG3 + AG1478 group were significantly increased (all $P < 0.05$). Compared with model group, the mRNA expression levels of DSG3, EGF and EGFR in nasal mucosa tissues of siRNA-DSG3 group and siRNA-DSG3 + AG1478 group were significantly decreased (all $P < 0.05$), while the mRNA expression levels of EGF and EGFR in nasal mucosa tissues of AG1478 group were significantly decreased (all $P < 0.05$), and there was no significant change in the DSG3 expression level ($P > 0.05$). There was no significant difference between model group and empty vector group (all $P > 0.05$). Compared with siRNA-

DSG3 group, the mRNA expression of DSG3 in AG1478 group was significantly increased ($P < 0.05$), while there was no significant difference in the mRNA expression of EGF and EGFR (all $P > 0.05$). However, there was no significant difference in the mRNA expression of DSG3 in siRNA-DSG3 + AG1478 group ($P > 0.05$), and the mRNA expression of EGF and EGFR were significantly decreased (all $P < 0.05$). The results showed that mRNA expression levels of DSG3, EGF and EGFR were increased in nasal mucosa tissues of AR mice. Silencing the DSG3 gene could inhibit the activation of the EGF signaling pathway.

3.5. Protein expression levels of DSG3, EGF, EGFR, and p-EGFR in nasal mucosa tissues of mice in each group

The protein expression levels of DSG3, EGF, EGFR and p-EGFR in epithelial tissues of the nasal mucosa of mice in each group were detected by western blot. See Fig. 5. Compared with control group, the protein expression levels of DSG3, EGF, EGFR and p-EGFR in the nasal mucosa tissues of mice in model group, empty vector group, siRNA-DSG3 group, the AG1478 group and siRNA-DSG3 + AG1478 group were significantly increased (all $P < 0.05$). Compared with model group, the protein expression levels of DSG3, EGF, EGFR and p-EGFR in the nasal mucosa tissues of siRNA-DSG3 group and siRNA-DSG3 + AG1478 group were significantly decreased (all $P < 0.05$), while the protein expression levels of EGF, EGFR and p-EGFR in the nasal mucosa tissues of AG1478 group were significantly decreased (all $P < 0.05$), and there was no significant change in DSG3 expression level ($P > 0.05$). There was no significant difference between model group and empty vector group (all $P > 0.05$). Compared with siRNA-DSG3 group, protein expression of DSG3 in AG1478 group was significantly increased ($P < 0.05$), and there was no significant difference in EGF EGFR and p-EGFR protein expression (all $P > 0.05$). There was no significant difference in the expression of DSG3 protein in siRNA-DSG3 + AG1478 group ($P > 0.05$), and the expression of EGF, EGFR and p-EGFR were significantly decreased (all $P < 0.05$). The results showed that the protein expression levels of DSG3, EGF, EGFR and p-EGFR were increased in the nasal mucosa tissues of AR mice. Silencing DSG3 gene could inhibit the activation of EGF signaling pathway.

3.6. Detection of apoptosis in nasal mucosa tissues of mice in each group

The results of TUNEL staining showed that compared with control group, the number of positive apoptotic cells in the nasal mucosa tissues of mice in model group, empty vector group, siRNA-DSG3 group, AG1478 group, and siRNA-DSG3 + AG1478 group was significantly

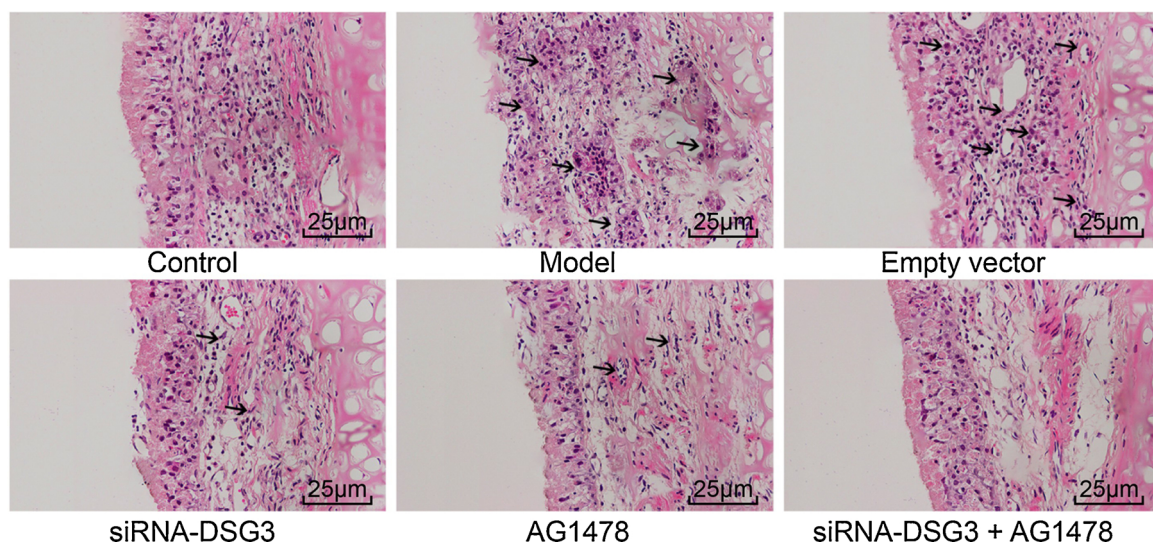


Fig. 2. HE staining detection of pathological features of nasal mucosa tissues in each group ($\times 400$). Scale bar = 25 μm .

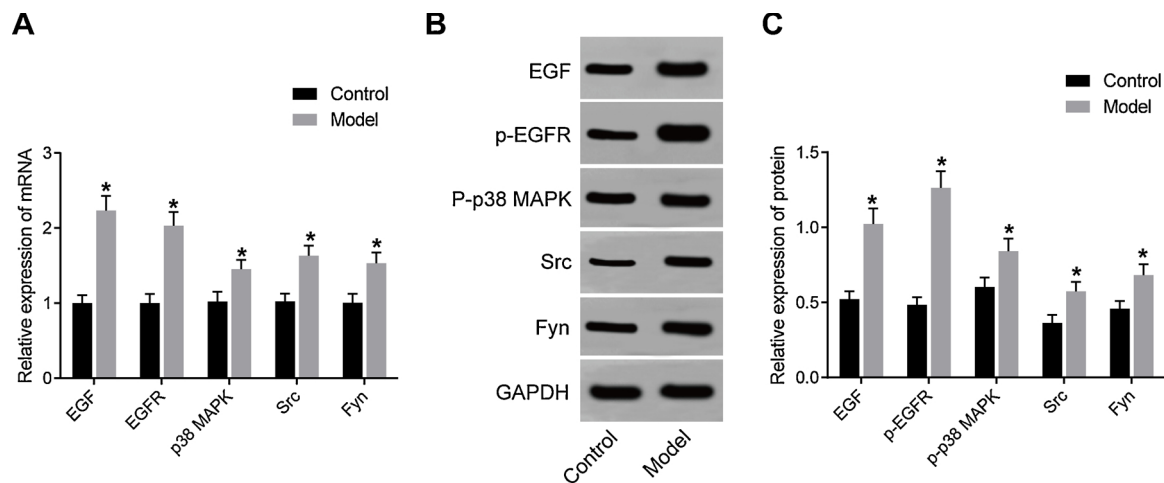


Fig. 3. qRT-PCR and western blot detection of mRNA and protein expression levels of EGF, EGFR, p38MAPK, Src and Fyn in nasal mucosa tissues of mice in each group qRT-PCR quantitative expression histogram. B. Western blot protein level band diagram. C. Western blot protein expression level histogram. Compared with control group, * $P < 0.05$. $n = 10$ in each group. The measurement data was expressed in the form of mean $\pm$ standard deviation ($\bar{x} \pm sd$), and it was used t -test. Each experiment was repeated three times.

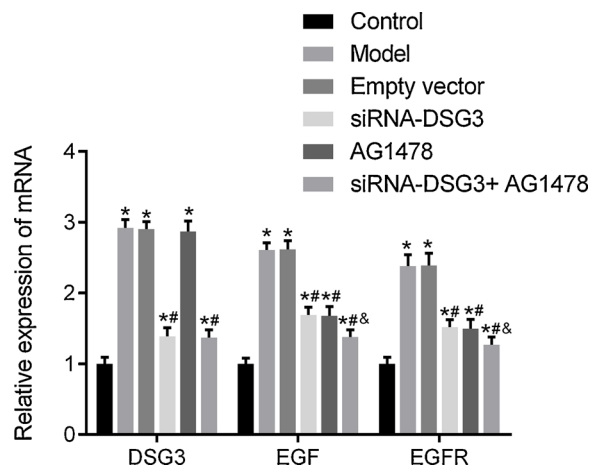


Fig. 4. qRT-PCR detection of mRNA expression of DSG3, EGF and EGFR in nasal mucosa tissues of mice in each group. Compared with control group, * $P < 0.05$; compared with model group, # $P < 0.05$; compared with siRNA-DSG3 group, & $P < 0.05$. DSG3: desmoglein 3; EGF/EGFR: epidermal growth factor/epidermal growth factor receptor.

increased, and the AI was significantly increased (all $P < 0.05$). See Fig. 6. Compared with model group, the number of positive apoptotic cells in the nasal mucosa tissues of mice in siRNA-DSG3 group, AG1478 group and siRNA-DSG3 + AG1478 group were significantly reduced, and the AI was significantly decreased (all $P < 0.05$). And the number of positive apoptotic cells in the nasal mucosa tissues of siRNA-DSG3 + AG1478 group was significantly lower than that of siRNA-DSG3 group and AG1478 group (both $P < 0.05$). There was no significant difference between model group and empty vector group (all $P > 0.05$). The results showed that silencing DSG3 gene or inhibiting the activation of EGF signaling pathway could inhibit the apoptosis of nasal mucosa cells in AR.

3.7. ELISA detection of the expression of EGF, EGFR, IgE, INF- γ , TNF- α , IL-2, IL-4 and IL-6 in serum of mice of each group

The levels of EGF, EGFR, INF- γ , TNF- α , IL-2, IgE, IL-4 and IL-6 in serum of mice were detected by ELISA method. The results showed that compared with control group, the levels of INF- γ and IL-2 in the serum of mice of model group, empty vector group, siRNA-DSG3 group, AG1478 group, and siRNA-DSG3+AG1478 group were significantly decreased, and the levels of EGF, EGFR, IgE, TNF- α , IL-4 and IL-6 in

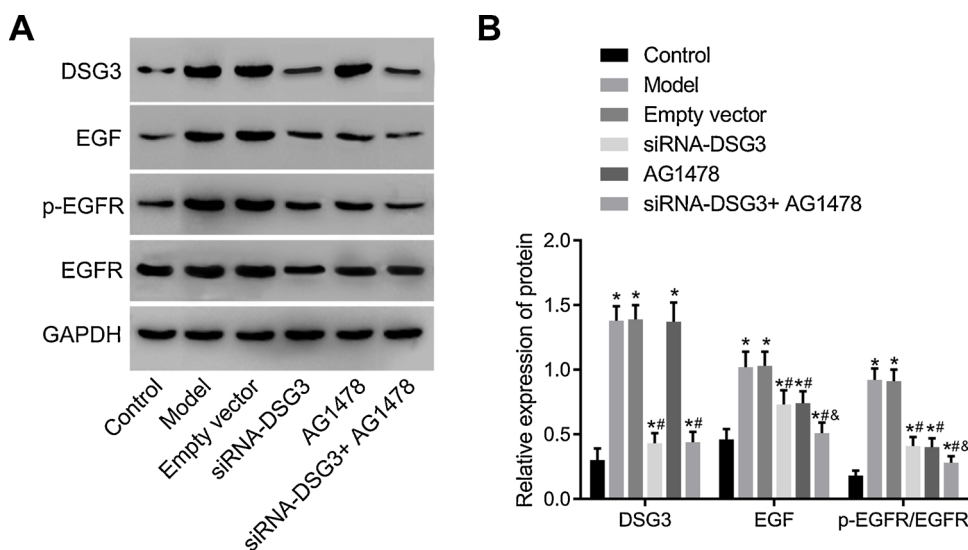


Fig. 5. Comparison of DSG3, EGF, EGFR p-EGFR protein levels in nasal mucosa tissues of mice in each group by western blot. A. Western blot was used to detect the protein level histogram of DSG3, EGF, EGFR and p-EGFR in nasal mucosa tissues of each group. B. The protein expression levels of DSG3, EGF, EGFR and p-EGFR were detected by western blot. Compared with control group, * $P < 0.05$; compared with model group, # $P < 0.05$; compared with siRNA-DSG3 group, & $P < 0.05$. DSG3: desmoglein 3; EGF/EGFR: epidermal growth factor/epidermal growth factor receptor.

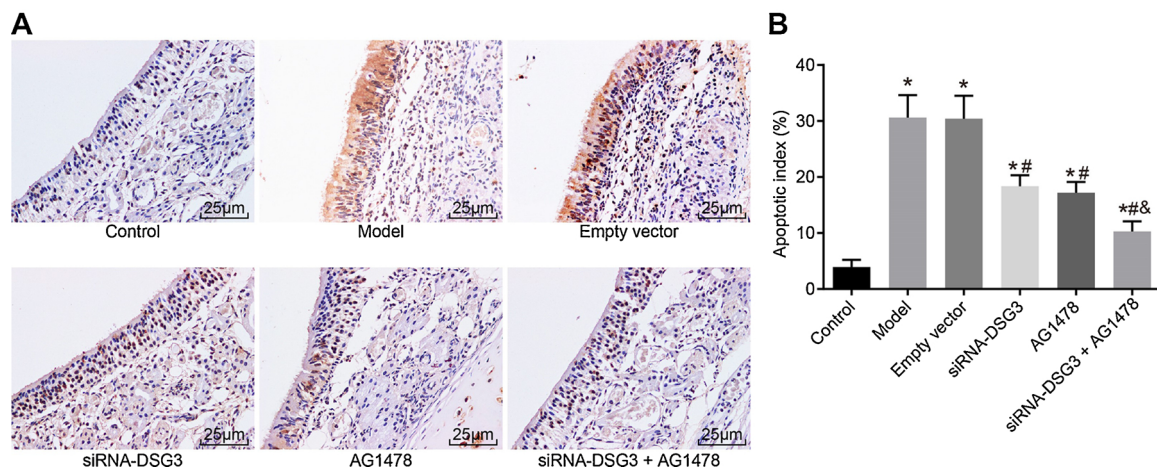


Fig. 6. Apoptosis of nasal mucosa tissues of mice in each group. A. TUNEL staining results of nasal mucosa tissues of mice in each group ($\times 400$). B. TUNEL staining was used to detect the AI of positive cells in the nasal mucosa tissues of mice in each group. Compared with control group, * $P < 0.05$; compared with model group, # $P < 0.05$; compared with siRNA-DSG3 group, & $P < 0.05$. Scale bar = 25 μm . AI: apoptotic index.

serum of mice were significantly increased (all $P < 0.05$). Compared with model group, the levels of IFN- γ and IL-2 in serum of mice were significantly increased in siRNA-DSG3 group, AG1478 group and siRNA-DSG3 + AG1478 group, and the levels of EGF, EGFR, IgE, TNF- α , IL-4 and IL-6 in serum of mice were significantly decreased (all $P < 0.05$). There was no significant difference between model group and empty vector group (all $P > 0.05$). The results showed that silencing DSG3 gene or inhibiting the activation of EGF signaling pathway could decrease levels of IgE, TNF- α , IL-4 and IL-6 in serum of mice and increase levels of IFN- γ and IL-2 in serum of mice. See Fig. 7.

3.8. Detection of red blood cells immune adhesion function in mice of each group by complement sensitization yeast hemagglutination method

The C₃b receptor rosette rate and Ic rosette rate of mice in each group were detected by complement sensitization yeast hemagglutination method. The results showed that compared with control group, the C₃b receptor rosette rate and the Ic rosette rate of model group,

empty vector group, siRNA-DSG3 group, AG1478 group, and siRNA-DSG3 + AG1478 group were significantly decreased (all $P < 0.05$). Compared with model group, the C₃b receptor rosette rate and the Ic rosette rate of siRNA-DSG3 group, AG1478 group, and siRNA-DSG3 + AG1478 group were significantly increased (all $P < 0.05$), and the C₃b receptor rosette rate and Ic rosette rate of siRNA-DSG3 + AG1478 group were significantly higher than those of siRNA-DSG3 group and AG1478 group (all $P < 0.05$). There was no significant difference between model group and empty vector group (all $P > 0.05$). The results showed that silencing DSG3 gene or inhibiting the activation of EGF signal pathway could enhance the immune adhesion function of red blood cells. See Fig. 8.

3.9. mRNA and protein expression levels of DSG3, EGF and EGFR in nasal mucosa tissues of normal mice and overexpressing DSG3 model mice

The results of qRT-PCR showed that compared with control group, the mRNA expression levels of DSG3, EGF and EGFR in nasal mucosa

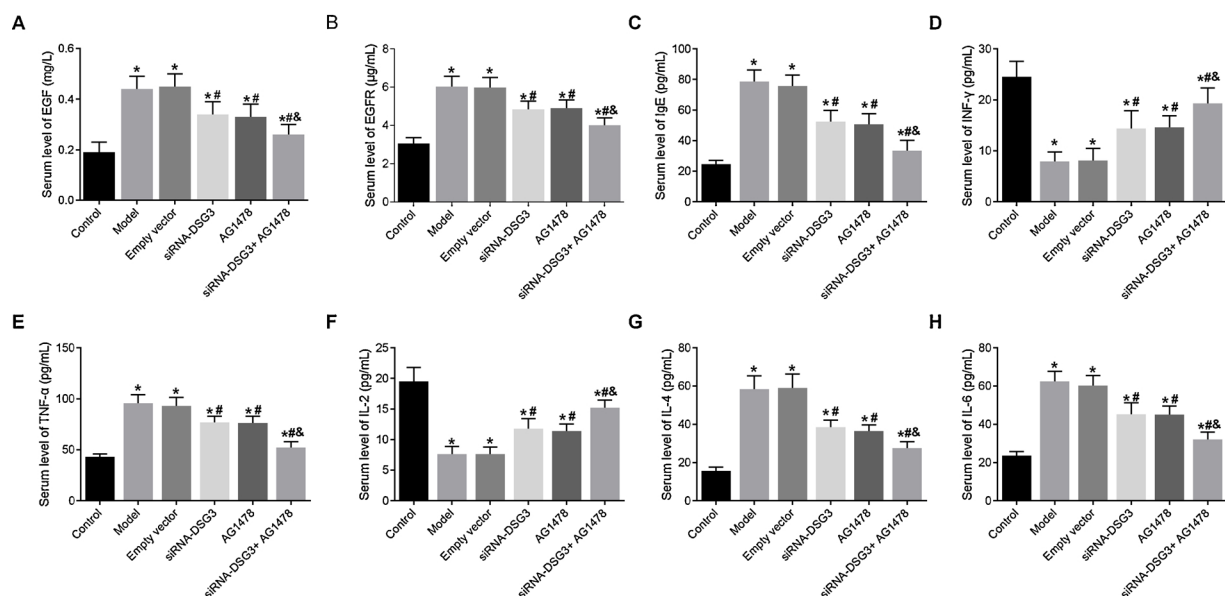


Fig. 7. ELISA detection of inflammatory factor levels in mice of each group. A. The EGF level of serum in mice of each group. B. The EGFR level of serum in mice of each group. C. The IgE level of serum in mice of each group. D. The INF- γ level of serum in mice of each group. E. The TNF- α level of serum in mice of each group. F. The IL-2 level of serum in mice of each group. G. The IL-4 level of serum in mice of each group. H. The IL-6 level of serum in mice of each group. Compared with control group, * $P < 0.05$; compared with model group, # $P < 0.05$; compared with siRNA-DSG3 group, & $P < 0.05$.

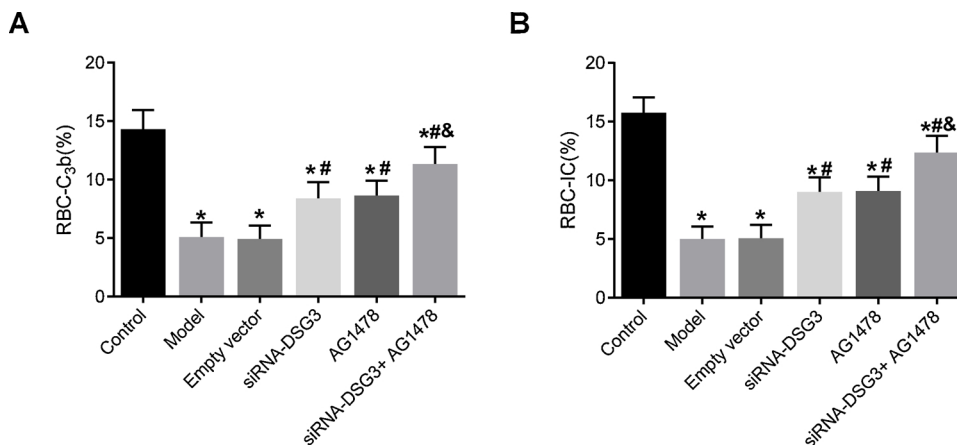


Fig. 8. Detection of red blood cells immune adhesion function in mice of each group by complement sensitization yeast hemagglutination method. A. C₃b receptor rosette rate in mice of each group. B. Ic rosette rate in mice of each group. Compared with control group, * $P < 0.05$; compared with model group, # $P < 0.05$; compared with siRNA-DSG3 group, & $P < 0.05$.

tissues of mice in oe-DSG3 group were significantly increased (both $P < 0.05$, Fig. 9A). The results of western blot showed that compared with control group, the protein expression levels of DSG3, EGF and p-EGFR in nasal mucosa tissues of mice in oe-DSG3 group were significantly increased (both $P < 0.05$, Fig. 9B and C).

4. Discussion

AR is mainly a nasal mucositis disease mediated by IgE after exposure to specific allergens, and its pathology may be related to the imbalance of immune cell secretion caused by the body after being stimulated by the allergens [19,20]. From the perspective of cellular molecules, it was found that DSG3 gene silencing could affect the inflammatory response of the nasal mucosa of AR by inhibiting the activation of EGFR/EGF signaling pathway, improve the immune adhesion function of red blood cells.

Firstly, we used qRT-PCR and western blot to detect the factors related to the pathway of AR in the nasal mucosa of mice in each group. Compared with control group, the expression of EGF/EGFR pathway-related factors in model group increased more significantly than other pathway-related factors. Therefore, we selected the EGF/EGFR pathway for study. Helper T cells (Thelper, Th) have been shown to play an important role in the regulation of immune response and the pathogenesis of immune-related diseases [21]. Th1 and Th2 cells can participate in the immune response by secreting corresponding cytokines [22]. Under normal circumstances, Th1 and Th2 cells have important protective effects on the human body, but once they and their secreted cytokines are unbalanced, they are very likely to cause a series of allergic diseases

[23]. For example, the occurrence of AR is directly related to the decrease of INF- γ and IL-2 secreted by Th1 cells and the increase of IgE, IL-4 and IL-6 secreted by Th2 cells [24]. These cytokines often affect the pathogenesis and progression of infectious diseases by participating in the cellular immune response [25,26]. IgE-mediated non-infectious reaction of nasal mucosa is an important cause of AR, and IL-4 and IL-6 also play important roles in humoral immune response and the pathogenesis of AR [27]. In this study, by detecting the expression of cytokines in the serum of mice in each group, it was found that silencing the DSG3 gene or inhibiting the activation of EGF signaling pathway could significantly decrease the expression levels of IgE, TNF- α , IL-4 and IL-6 in the serum of AR mice, and promote the increasing of expression levels of INF- γ and IL-2. The combined use of DSG3 gene and EGF signaling pathway inhibition is better. Increased expression of IgE and inflammatory factors TNF- α and IL-4 and IL-6 are important inflammatory factors in the development of AR, and the level of expression is closely related to the severity of the disease [28]. However, INF- γ and IL-2 have been shown to activate macrophages and play an active role in the regulation of immune system function [29,30]. The results of this study also confirmed that silencing DSG3 and inhibiting EGFR/EGF signaling pathway could increase the expression of INF- γ and IL-2, suggesting that it plays a positive role in the regulation of AR immune factors.

In AR, epithelial organelle damage is aggravated by epidermal cell secretion, neurotransmitters and local humoral factors, and cell apoptosis is an important way to maintain normal cell renewal and epithelial stability.

Under pathological conditions such as AR, cell apoptosis is

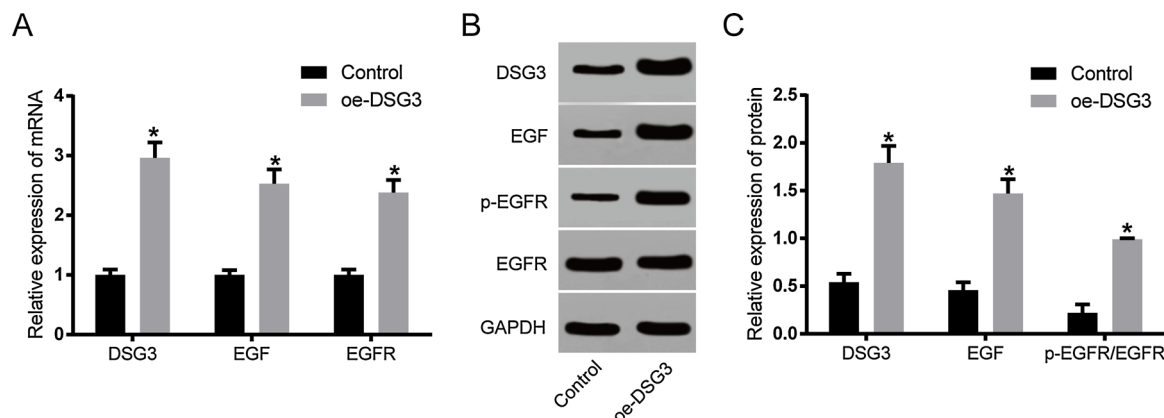


Fig. 9. qRT-PCR and western blot detection of mRNA and protein expression levels of DSG3, EGF, and EGFR in nasal mucosa tissues of mice in control group and oe-DSG3 group. A. qRT-PCR detection of mRNA expression. B. Western blot detection of protein level band diagram. C. Western blot protein expression histogram. Compared with control group, * $P < 0.05$. $n = 10$ in each group. The measurement data was expressed in the form of mean $\pm$ standard deviation ($\bar{x} \pm sd$), and it was used t -test. Each experiment was repeated three times.

significantly reduced, which may be related to inflammatory reaction conditions; if the inflammation is not controlled in time, chronic hyperplasia of the mucosa and other diseases are easily triggered [31]. In the experiments of *in vivo*, Tunel staining was used to observe the cell apoptosis in each group, compared with model group, the cell apoptosis of in siRNA-DSG3 group and AG1478 group was significantly increased, and the number of apoptosis cells in siRNA-DSG3 + AG1478 group was the most in AR model mice of each group. This suggesting that DSG3 gene silencing or inhibiting the activation of EGFR/EGF signaling pathway can promote the apoptosis of AR nasal mucosa cells and respond to the excessive inflammatory response in AR. Immunological studies have confirmed that red blood cells are an important part of human immune cells and can participate in the regulation of human immune function [32]. At the same time, red blood cells also have the function of recognizing, concentrating, promoting phagocytosis, presenting antigens and enhancing immune function of T lymphocytes [33]. The reduction of red blood cells immune adhesion function is considered to be an important basis for the pathogenesis of various immune-related diseases including AR [34]. In this study, the red blood cells immune adhesion function of the cells in each group was detected, and it was found that silencing DSG3 gene or inhibiting the EGFR/EGF signaling pathway is of great importance for improving the immune function of red blood cells.

By confirming the relationship between DSG3 gene and EGFR/EGF signaling pathway through qRT-PCR and western blot methods, it is found that DSG3 gene is related to the activation of EGFR/EGF signaling pathway. This suggesting that the regulation of AR inflammatory response and immune function by DSG3 gene silencing are mainly achieved by regulating the function of its downstream EGFR/EGF pathway. However, whether the protective effect of DSG3 gene silencing on AR is affected by other potential factors in this study still needs further experimental confirmation.

5. Conclusions

In conclusion, this study confirmed that DSG3 gene silencing could inhibit the activation of EGFR/EGF signaling pathway, thereby alleviating the inflammation of AR nasal mucosa, and enhancing the immune adherence function of red blood cells, which has a protective effect on the pathogenesis of AR. DSG3 gene is expected to become an important target for AR treatment.

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Declaration of Competing Interest

Authors declared that they have no conflict of interest.

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