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Down-regulated Rac1 promotes apoptosis and inhibits the clearance of apoptotic cells in airway epithelial cells, which may be associated with airway hyper-responsiveness in asthma

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Running title: Low expression of Rac1 may lead to airway hyper-responsiveness

Abstract

The accumulation of airway apoptotic cells may be an important factor causing airway hyper-responsiveness. Whether the apoptotic cells can be promptly removed is related to the occurrence and course of asthma. In recent years, studies have shown that Rac1 is involved in many cellular biological activities including the formation and elimination of apoptotic cells. In this study, based on analysis of airway local cells and related factors in asthmatic mice, we evaluated the expression of Rac1 in airway epithelial cells or phagocytes, and analyzed its relationship with the incidence of apoptosis or scavenging of apoptotic cells. Our data showed that the expression level of Rac1 in asthmatic mice decreased significantly, while the expression of IL-33 increased obviously. The airway epithelial cell line was stimulated by curcumin at 50 μ mol/L for 24-48 hours, more than 50% of the cells were apoptotic, of which about 20% were late apoptosis. Rac1 inhibitor (NSC23766) can enhance the apoptosis effect. In addition, the ability of phagocytosis and migration in the epithelial cells or macrophages was increased following the application of Rac1 inhibitors or specific siRNA in a dose-dependent manner, and the expression level of IL-33 was simultaneously increased after blocking Rac1. It is suggested that the down-regulation of Rac1 in asthma may contribute to the apoptosis of airway epithelial cells and affect the clearance of apoptotic cells, which will lead to the aggregation of the apoptotic cells in the respiratory tract and participate in airway hyper-responsiveness.

Key words: Rac1; airway hyper-responsiveness; apoptotic cells; airway epithelial cells; asthma; mice

Background

Asthma is one of the most common chronic inflammatory airway diseases along with constriction of airway smooth muscle, hyper secretion of mucus, edema and airway hyper-responsiveness (AHR) and thickening of the basement membrane underlying the airway epithelium. Lack of treatment and low compliance with medications are the greatest challenges in managing the disease, since these increase the risks of exacerbations leading to emergency department visits, hospital admissions, and consequently, to high costs to the public services, in addition to significantly lowering the quality of life of those involved. Hence, to investigate new exact mechanisms and molecular pathways activated in asthma is necessary in developing novel treatment measures and therapeutic drugs in asthma [1].

Previous studies have demonstrated that the repair process of airway epithelium in asthma was defective fundamentally, and abnormal airway epithelial shedding was observed in asthmatic patients. Rac1 is a member of the Rho family and widely distributed in various tissues. It participates in cell motility, migration and metastasis, cell actin reorganization, cell proliferation and apoptosis. Studies have shown that Rac1 overexpresses [2,3] in many lung cancer, breast cancer, colon cancer, gastric cancer, pancreatic cancer, head tumor and other tumor tissues. However, the role of Rac1 in immune inflammation is not yet clear. Recent studies have shown that airway epithelial cells phagocytose inflammatory cells and secrete the effective anti-inflammatory cytokine depend on the signal transduction of Rac1, loss of Rac1 expression in airway epithelial cells can lead to a defect on phagocytosis and produce inflammatory cytokines (possibly including IL-33) in epithelial cells [4-6]. Rac1 expression in airway epithelial cells is closely related to a variety of airway inflammation and AHR, the down-regulation of its expression will contribute to increase the expression of IL-33, which promotes ILC2s polarization [7]. It is speculated that the apoptosis of airway epithelial cells and the timely clearance of apoptotic cells may relate to the occurrence of airway hyper responsiveness. The low expression of Rac1 in asthma may not only affect the activity and power of professional or non-professional phagocytes, but also involve the apoptosis of airway epithelial cells. In this study, based on analysis of local cells and related factors in asthmatic mice, we evaluated the expression of Rac1 in airway epithelial cells or phagocytes, and analyzed its relationship with the incidence of apoptosis or scavenging of apoptotic cells.

Materials and methods

Animals and asthma model induction

Female Balb/c mice (6 weeks old), weighing 18 ± 2 g, were purchased from the laboratory animal center of Yangzhou University (Yangzhou, China). Mice were housed in plastic cages with sterilized wood-chip bedding, bred in animal rooms kept at a temperature of $23\pm 2^{\circ}\text{C}$ and a relative humidity of $55\%\pm 10\%$ with a 12-hour light-dark cycle. They had free access to tap water and normal diet. The mouse model of allergic airway inflammation was established as described by Wang et al [8] with slight modification. Briefly, the mice were sensitized by intraperitoneal injection of $50\mu\text{g}$ OVA protein and 2 mg aluminum hydroxide gel in saline. A second sensitization was given 10 days after the initial sensitization. On day 22 after initial sensitization, mice were placed in a Plexiglas chamber linked to an ultrasonic nebulizer and challenged with aerosolized OVA solution (10 mg/mL in saline) for 60 minutes. The provocation was performed once a day for 5 consecutive days and then the mice were assessed for allergic inflammation of the lungs 24 hours after the last aerosol exposure. All experimental procedures were approved by the Jiangsu University Institutional Committee on the Use of Animals for Research and Teaching.

Cell culture and exogenous gene transferring

Airway epithelial cell line A549 was purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's modification of Eagle's medium (DMEM) containing 10% fetal bovine serum (Gibco, Grand Island, USA) at 37°C with 5% CO_2 atmosphere. Macrophage cell line RAW264.7 (purchased from the Cell bank of Shanghai, Chinese Academy of Sciences) was cultured in 1640 medium containing 10% fetal bovine serum (Gibco, Grand Island, USA) at 37°C with 5% CO_2 atmosphere. The plasmid pCDNA3.1-Rac1 (prepared in our laboratory) and siRNAs (including Rac1 homo-324 and 626, Suzhou GenePharma, China) were transferred into RAW264.7 or A549 cell line by liposome according to our previous research [9].

Induction of apoptosis

Curcumin was selected as a stimulating factor to induce apoptosis of airway epithelial cell (A549, an alveolar basal epithelial cell line). Cells were inoculated in 24-well cell culture plate containing DMEM medium with 10% fetal bovine serum (FBS), and 50 $\mu\text{mol/L}$ curcumin was added. The cells were incubated for 48 hours. The cells were collected and washed two times with PBS and $1 \times$ binding buffer respectively, and resuspend in binding buffer. Add 5 μl Annexin V and incubated around 15 min at room temperature. After washing with $1 \times$ binding buffer, flow cytometry analysis was performed within 4 hours.

RNA Extraction and Quantitative Real-time PCR

Total RNA was extracted by guanidinium thiocyanate phenol chloroform method, 500 ng RNA was reverse transcribed using PrimeScript^{RT} reagent Kit (TAKARA), according to the manufacturer's instructions. On the basis of Genebank sequences, the primers used in this study were designed by Premier 5.0 software and synthesized by Shanghai Invitrogen. The primers used were as follows: IL-33 forward:

5'-TCCCAACAGAAGACCAAAGAA-3', reverse: 5'-CCGTGGATAGGCAGAGAAGT-3';

Rac1 forward: 5'-GTAAACCTGCCTGCTCATCA-3',

reverse: 5'-GGACGCAATTCATAATCTTC-3'; IL-1 β forward:

5'-CCCTGCAGCTGGAGAGTGTGGA-3', reverse: 5'-CTGAGCGACCTGTCTTGGCCG-3'

; sphk-1 forward: 5'-GGAACCAGTAGAATGCCCTC-3',

reverse: 5'-GGTTCTTCCGTTCGGTGAGT-3'; β -actin forward:

5'-ATGGAAATGGGGAAGATGGTC-3', reverse: 5'-GCGGGGAGGGTGTGAACT-3'.

Quantitative Real-time PCR (qRT-PCR) was conducted in a SYBR^R Premix Ex TaqTM (TAKARA) according to the manufacturer's instructions. Fold changes in the expression of each objective gene relative to β -actin were calculated based on the threshold cycle (Ct). All samples were performed in triplicate.

Flow cytometry analysis

Flow cytometry was used to detect cell apoptosis. Curcumin-induced apoptotic cells were stained with Annexin V and PI double staining method using Annexin V- FITC/PI apoptosis kit (BioVision). In brief, the cells induced by Curcumin were washed with PBS and Binding buffer respectively, and then suspended with 100 μ L Binding buffer. 5 μ L Annexin V was added into cell suspension, place at room temperature for 15 min, washed with 500 μ L Binding buffer, and re-suspended with 200 μ L Binding buffer, added 20 μ L PI, and analyzed by flow cytometry within 4 hours. Meanwhile, Hoechst nuclear staining was also used to observe the morphology of apoptotic cells by fluorescence microscopy according to the specific standard procedure (the working concentration of Hoechst 33342 is 2 μ g/ml).

Cell phagocytosis: A549 cells were selected and seeded in a 6-well cell culture plate at 4×10^5 cells per well, 5% CO₂ and incubated overnight at 37 $^{\circ}$ C, and then the apoptotic cells which induced for 48 hours were collected and added to the culture plate. The mixed cells were incubated at 37 $^{\circ}$ C for another 90 min, and analyzed by flow cytometer and qRT-PCR.

Changes of expression of CD80, CD86 and MHC II on RAW264.7 cell (a macrophage cell line) were analyzed by flow cytometry. 500ng/mL LPS was used to stimulate 24h in each group (at the same time, a control group without LPS stimulation was established). The cells were washed with PBS for two times, then PE-conjugated anti-mouse CD80, CD86 and MHC II antibodies (eBioscience, USA) were added respectively. The staining system of 100 - L was stained at 4 $^{\circ}$ C for 30 min and washed with PBS for two times. The isotype control antibody was used in all cases. For FACS phenotype analysis, data were acquired on an Accuri C6 (BD company) and analyzed with FlowJo software (TreeStar, Inc.). The experiment was repeated 3 times.

Transwell migration and cell proliferation analysis

Cells (1×10^5 /well) were plated into the top chamber and DMEM medium containing 10% FBS and 500ng/mL LPS was placed into the bottom chamber. After incubation at 37 $^{\circ}$ C in 5% CO₂ for 12 h, the cells remaining at the upper surface of the membrane were removed with a cotton swab. The cells that migrated through the 8 μ m sized pores and adhered to the lower surface of the membrane were fixed with 4% paraformaldehyde, stained with crystal violet and photographed.

CCK-8 assay for cell proliferation: Cells (8×10^3 /well) were plated into 96 well plates, after incubation at 37° C in 5% CO₂ for 24 h, the supernatant was discarded and fresh FBS-free 1640 medium and 10 μl CCK-8 reagent were added. Then the mixture was incubated for another 2 hours. The absorbance at 450 nm (OD 450) was read by enzyme labeling instrument, the intensity of cell proliferation was evaluated by OD 450.

ELISA for serum IgE and IL-33

The serum IgE and IL-33 were measured by ELISA kit following the manufacturer's protocols. All samples were measured in triplicate, and the mean concentration was calculated from the standard curve.

Western blotting

The cells were homogenized and lysed in RIPA buffer supplemented with proteinase inhibitors. Equal amounts of total protein were loaded and separated on 12% SDS-polyacrylamide gel. Following electrophoresis, the proteins were transferred to a PVDF (polyvinylidene difluoride) membrane (Millipore, USA), blocked in 5% (w/v) non-fat milk and incubated with the primary antibodies. Membranes were incubated with monoclonal antibody against GAPDH (Rabbit anti mouse, Abcam, USA) and Rac1 (Rabbit anti mouse, Abcam, USA) at 4°C overnight. The membrane was washed with Tris-buffered saline/Tween (TBS/T) for three times and incubated with the secondary antibodies (Sheep anti rabbit HRP-IgG, Abcam, USA) at 37°C for 1 h. The signals were visualized by using Luminata crescendo western horseradish peroxidase (HRP) substrate (GE corporation, USA).

Statistical analysis

All data were shown as means ± standard deviation (SD). The statistically significant differences between groups were assessed by analysis of variance (ANOVA) or t-test using Prism software (GraphPad, San Diego, USA). P value <0.05 was considered significant.

Results

Decreased Rac1 was accompanied by increased IL-33 in asthma

The mouse model of allergic airway inflammation was established, which showed the elevated serum IgE level and obvious airway inflammation (Figure1). The analysis of asthma model showed that the expression of Rac1 in peripheral blood mononuclear cells, lung tissues and alveolar lavage fluid was significantly decreased, while the expression of IL-33 in peripheral blood and lung tissues was increased. These results were consistent with our earlier studies [10,11], suggesting that Rac1 expression disorder was closely related to the occurrence of asthma, and was associated with up-regulated IL-33, which activated the activity of ILC2s. What is the mechanism of its involvement in asthma? We have done further research in the following research.

Down regulation of Rac1 expression promoted the apoptosis of airway epithelial cells in vitro

Formation and clearance of apoptotic cells in the airways may be a potential factor for airway hyper-responsiveness in asthma. In this study, we analyzed the relationship between the expression of Rac1 and apoptosis in the airway epithelial cell line A549 in vitro, so as to explore the factors and possible mechanisms of airway hypertension in asthma, and to find new and possible intervention targets for asthma control. Curcumin was used to induce apoptosis in airway epithelial cells and the results showed in Figure2. Increased ratio of apoptotic cells was found in the presence of Rac1 inhibitor (NSC23766). Our data also showed that there was a certain phagocytosis of apoptotic cells in airway epithelial cells, but it was inhibited by NSC23766. Meanwhile, the results from qRT-PCR showed that the expression level of IL-33 mRNA was upregulated under the condition of Rac1 inhibitor existence, but the expression of Rac1 had not changed when airway epithelial cells swallowed apoptotic cells (Figure2).

Down regulation of Rac1 expression weakened the clearance of phagocytes against apoptotic cells in the airway epithelial cell

Rac1 specific small interfering RNA downregulated the Rac1 expression level by silencing Rac1 gene, while Rac1 inhibitor NSC23766 blocked its binding to guanine nucleotide purine exchange factor (GEF). In this trial, we used siRNA to inhibit the expression of Rac1 in the airway epithelial cell line and observed their scavenging effect on homogenous apoptotic cells. Our data indicated that the expression of Rac1 mRNA was down-regulated, and the phagocytosis of apoptotic cells was significantly inhibited in the Rac1 siRNA-transfected A549 cells. In addition, the abilities of cell proliferation, migration and the expression of SphK1 mRNA were decreased, but the expression level of IL-33 was increased in siRNA-transfected A549 which engulfed apoptotic cells (Figure3).

In combination with the above results, the low expression of Rac1 in asthma was conducive to the apoptosis of respiratory epithelial cells, but not conducive to the clearance of the apoptotic cells by epithelial cells.

Effect of Rac1- siRNA on antigen presentation of macrophage

The results showed that the expression of Rac1 mRNA and protein were significantly up-regulated in Rac1-transfected RAW264.7 cells, but the results were the opposite in siRNA-transfected cells. There were no significant changes in the expression of CD80, CD86 and MHC-II in Rac1-transfected RAW264.7 cells. However, these three kinds of membrane molecules could be up-regulated by Rac1 inhibitors or specific siRNA. The mRNA and protein levels of IL-1 β in supernatants of Rac1-transfected RAW264.7 cells were lower than that in siRNA-transfected cells (Figure4). It indicated that the positive regulation of Rac1 may contribute to the proliferation of RAW264.7 cells, which will further facilitate the phagocytic cells to scavenge the apoptotic cells. On the contrary, inhibiting the expression of Rac1 affected the phagocytosis of macrophages, but it was beneficial to their antigen presentation (Figure4).

Discussion

The RhoGTP enzyme family, composed of Ras-related C3 botulinum toxin substrate 1 (Rac1) and RhoA, has been considered to be involved in cytoskeletal recombination, as a crucial regulator of actin cytoskeleton dynamics and endothelial contractions. Deregulation of these Rho-GTPases may result in a disorder of the actin cytoskeleton and may further impact the integrity of the endothelial barrier. Rac1 aggregates on the plasma membrane to form membrane processes, which are beneficial to the phagocytic function of macrophages [12]. Asthma has long been a focus of researchers as a chronic inflammatory disease. Rac1 is widely expressed in airway epithelial cells and phagocytes. What is the role of Rac1 in the pathogenesis and development of asthma, it remains to be further studied. Airway hyper-responsiveness is an important pathological feature of asthma, and the airway epithelial cells, intrinsic lymphocytes and dendritic cells participate in the process. In the present study, our data showed that the expression of Rac1 in asthmatic mice was significantly lower than that in the control group, while the expression of IL-33 was significantly higher, and Rac1 was negatively correlated with the expression of these cytokines. Studies have shown that when siRNA knocks out Rac1 in bronchial epithelial cells, IL-33 production increases significantly [13]. Although the release of IL-33 is a complex process regulated by allostrogens and enzymes, these studies suggest that the release of IL-33 may be regulated by a Rac1-dependent mechanism, it is consistent with our current research findings.

Previous studies have shown that Rac1 is overexpressed in lung cancer, breast cancer, colon cancer, gastric cancer, pancreatic cancer, head cancer and other tumors [14-18]. With the development of Rac1 in the field of cancer research, it is not limited to its important regulatory role in cell migration and cancer deterioration. The effect of Rac1 on tumor cell apoptosis has been gradually found and attracted attention [19,20]. It has been pointed out that the expression of Rac1 in leukemia cells HL-60 is higher than that in normal cells, and the apoptosis rate of HL-60 cells is lower than that of normal cells under specific treatment [21]. After adding Rac1 inhibitor or using siRNA to knock out Rac1, the same apoptosis experiment showed that the apoptosis rate of HL-60 cells increased significantly, suggesting that Rac1 played a negative role in the apoptosis of HL-60 cells. A similar phenomenon has also been found in breast cancer and colon cancer. In other chronic inflammatory diseases such as asthma, the mechanism of apoptosis regulation remains to be further explored. A549 cell line is derived from human alveolar basal epithelial cells, and our previous studies have shown that A549 cell line express similar levels of Rac1 as other bronchial epithelial cells.

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In the present study, we found that the expression of Rac1 was also closely related to the apoptosis in the airway epithelial cell line A549 in vitro. Inhibiting the expression of Rac1 in airway epithelial cells promoted the early and late apoptotic cells frequency and decreased the expression of SphK1 as an inhibitor of apoptosis, it suggested that asthmatic respiratory tract with low expression of Rac1 might be involved in airway obstruction due to the increase of airway apoptosis cells. We hypothesized that the production of apoptotic airway cells is a physiological process, but if too many apoptotic cells could not be cleared in time, it may lead to airway obstruction and other pathological changes. In recent years, studies have shown that the clearance effect of airway macrophages on apoptotic cells is helpful to alleviate lung inflammation. It has been reported that 10% cigarette smoke extract can inhibit the membrane localization of Rac1 and the ability of RAW264.7 cells to phagocytose microspheres. Similarly, in the lung tissues of mice treated with cigarette smoke extract, the localization of Rac1 membrane of airway macrophages was inhibited. In this study, we used Rac1 siRNA to inhibit the expression of Rac1 in the airway epithelial cell line A549 and observed their scavenging effect on homogenous apoptotic cells. Our data indicated that the expression of Rac1 mRNA was down-regulated, and the phagocytosis of apoptotic cells was significantly inhibited. In addition, the abilities of cell proliferation and migration were decreased, but the expression level of IL-33 was increased in siRNA-transfected A549 which engulfed apoptotic cells.

Immune balance is just like the axis of immune inflammation injury disease and tumor regulation. Immune imbalance caused by excessive up-regulation or down-regulation may be an important factor in the occurrence and development of these two diseases. Recent studies show that Rac1 can participate in immune regulation and affect the occurrence and development of immune-related diseases. We preliminarily discussed the Rac1 expression on macrophage cell line RAW264.7 and its effect on antigen presentation, the results indicated the CD80, CD86 and MHC-II in RAW264.7 cells could be up-regulated by Rac1 inhibitors or specific siRNA. The mRNA and protein levels of IL-1 β in supernatants of Rac1-transfected RAW264.7 cells were lower than that in siRNA-transfected cells. Macrophages are traditional and important antigen presenting cells, and their biological functions play an important role in both innate and adaptive immune responses [22]. Rac1 has a wide range of expression profiles, we further corroborated the expression level of Rac1 in respiratory epithelial cells and macrophages was closely related to airway hyper-responsiveness. It was not only related to the apoptosis and clearance of epithelial cells

in airway inflammation, but also affected the changes of macrophages or dendritic cells as antigen presenting cells. We speculated that this effect can be attributable to the fact that increased allergenic presenting ability in asthma, it may be partly due to the promotion of ILC2s and Th2 cell activity.

In conclusion, the increased expression level of Rac1 could promote the proliferation, migration and phagocytosis of apoptotic cells in airway epithelial cells. The inhibition of Rac1 enhanced the epithelial cell apoptosis and IL-33 production, reduced SphK1 expression in epithelial cell, and also facilitated the expression of CD80, CD86 and MHC-II in macrophages. In addition, Exogenous Rac1 has a negative regulatory effect on IL-1 β expression in macrophages. On the basis of these result, we hypothesized that decreased active Rac1 could contribute to the increased airway hyper-responsiveness observed in asthma (Figure 5), it also pointed out the important role of Rac1 as a therapeutic target to improve airway hyper-responsiveness in asthma.

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Competing interests

The authors declare that they have no competing interests.

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Legends

Figure 1 Expression levels of Rac1 and IL-33 in asthmatic models

Asthma mice were induced, its pathological features and related cytokines were detected. A: H&E staining was performed on lung tissue sections and showed a greater number of inflammatory cells infiltration in OVA-challenged mice compared with control. B and C: The plasma levels of OVA-specific IgE and total IgE in each group evaluated by ELISA. D-F: The expression of Rac1 from different tissue sites was revealed respectively, the results showed that the expression levels of Rac1 in peripheral blood, lung tissue and HALF were significantly lower in asthmatic models than that in control mice. G: Showing elevated plasma IL-33 detected by ELISA in asthmatic mice. F: Obviously up-regulated IL-33 was detected by RT-PCR in lung tissue of asthmatic mice. Data are presented as mean $\pm$ SD, * $p < 0.005$; ** $p < 0.001$ compared with control.

Figure 2 Rac1 expression affected apoptosis and clearance of apoptotic cells in airway epithelial cell line A549

A: Curcumin induced apoptosis in airway epithelial cell line A549. Flow cytometry analysis showed that the total apoptotic rate (including early and late apoptotic cells frequency) induced by Curcumin increased significantly when Rac1 inhibitor was added into the reaction system. B: Hoechst staining results of apoptotic cells. C: Flow cytometry analysis of apoptotic phagocytosis by A549 cells at three different times, the phagocytosis increased with the prolongation of phagocytosis time in A549 cells. D: Histogram of phagocytosis of apoptotic cells. E: The results of apoptotic phagocytosis observed by fluorescence microscopy (E). F: Inhibitory effects of NSC23766 on phagocytosis of apoptotic cells. G and H: The qRT-PCR analysis results, unchanged Rac1 expression (G) and increased expression of IL-33 (H) in epithelial cells devouring apoptotic cells. Data are presented as mean $\pm$ SD, * $p < 0.005$; ** $p < 0.001$ compared with control.

Figure 3 Effects of Rac1-siRNA or inhibitor on biological actions of airway epithelial cell line A549

Two kinds of siRNA (Rac1 homo-324 and 626) were selected to influence the biological function of A549 cells. A: Under the influence of Rac1-siRNA, the expression level of IL-33 in A549 cells was significantly reduced, especially under the influence of Rac1 homo-626. Therefore, Rac1-homo 626 was selected for the following studies. B and C: Rac1-homo 626 significantly inhibited the phagocytosis of apoptotic cells by epithelial cell line A549. B, a histogram for phagocytosis; C, showing the results of flow cytometry analysis. D: Inhibitor NSC23766 promoted the expression of IL-33 in the epithelial cells devouring apoptotic cells. E: Exogenous Rac1 gene promoted the expression of sphK1 in the epithelial cells, while Rac1-homo 262 and NSC23766 inhibited its expression. F: The results of migration ability in the epithelial cell line A549, it was significantly enhanced in exogenous Rac1 gene carried cells, and decreased in siRNA group or NSC23766 group. NC indicates control cells, Rac1 means the cells which carried exogenous Rac1 gene. Data are presented as mean $\pm$ SD, * $p < 0.005$; ** $p < 0.001$; *** $p < 0.0001$ compared with control.

Figure 4 Rac1 expression positively regulated the ability of antigen presentation in macrophage cell line RAW264.7

A: High expression of Rac1 mRNA and protein in phagocytic cell line RAW264.7 carrying Rac1 gene. B: Rac1-siRNA inhibited Rac1 expression in mRNA and protein level. C: Effect of Rac1 on proliferation of RAW264.7 cells (CCK-8 method). D: The results of cell membrane molecules CD80, CD86 and MHC II analyzed by flow cytometry, three kinds of molecules could be positively regulated by Rac1 inhibitors or specific siRNA. E-G: Histogram of flow analysis results in each group. H: IL-1mRNA measured by RT-PCR. I: Results of IL-1 protein detected by ELISA. NC: control cells, Rac1: the cells carrying exogenous Rac1 gene, RAW: RAW264.7 cell line, NSC50 μ M = 50 μ M NSC23766, NSC100 μ M = 100 μ M NSC23766. Data are presented as mean $\pm$ SD, * $p < 0.005$, ** $p < 0.001$ compared with control.

Figure 5 Possible mechanisms of airway hyper-responsiveness induced by low expression of Rac1 in asthma









