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Involvement of Claudin-1 in Thrombin Promoting Airway Remodeling in Asthma

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Keywords:

Claudin-1; Thrombin; Airway remodeling; Asthma

Abbreviations:

ASMC: Airway Smooth Muscle Cells; TJs: Tight Junctions; EMT: Epithelial-Mesenchymal Transformation; OVA: Ovalbumin; TGF-β: Transforming Growth Factor; EGFR: Epidermal Growth Factor Receptor; AHR: Airway Hyper Responsiveness; AJ: Adhesion Junctions; MCP-1: Monocyte Chemotaxis Protein-1.

1. Abstract

Claudin-1 is an important member of the cellular tight junction protein, which is very important to barrier function. But some studies have shown that Claudin-1 is associated with cell proliferation, so its role in asthma gets more and more attention. Asthma is associated with clotting system in the lungs. Thrombin is thought to be associated with pro-inflammatory, pro-proliferative and pro-migratory activities. Also, thrombin can result in the disintegration of Claudin-1 from the tight junction protein complex. Therefore, we speculated that thrombin may interact with Claudin-1 in the lung tissue of asthma. Thirty female mice were randomly divided into 3 groups: normal control group, asthma group, thrombin group. The expression of claudin-1 was observed by immunofluorescence and RT-PCR. Mouse airway smooth muscle cells were cultured in vitro, and after Claudin-1 siRNA was transfected, CCK8 was used to detect the change of proliferation ability, and transwell was used to detect the change of cell migration ability. Different drug interventions were given to mouse airway smooth muscle cells, and the expression of claudin-1 was detected by western blot.

1.1. Conclusion

Claudin-1 is related to the proliferation and migration of ASMCs. Restricting the expression of Claudin-1 in ASMCs can weaken airway remodeling caused by thrombin in asthma mice.

2. Introduction

The bronchial epithelium of asthma is destroyed and disintegrated. Epithelial shedding is the main characteristic of asthma. Airway epithelial cells exfoliation and goblet cells lose their cilia. The result of these changes is the destruction of the integrity of the tight junction of the epithelium and the repairment of the damaged tissue after injury [1]. Tight junctions include at least four types, called claudins, occludins, junction adhesion molecules and detrital [2]. Claudin-1 is the main structural component of TJs [3,4]. Repeated airway inflammation in asthmatic patients results in varying degrees of damage to claudin-1, the tight junction protein of epithelial cells, and destruction of epithelial integrity, which in turn increases cell permeability, stimulates chronic inflammation, and ultimately leads to airway remodeling. Asthmatic epithelial cells establish a series of repair processes that play a key role in driving airway remodeling. Cell migration, the formation of temporary barrier, leading to epithelial-mesenchymal transformation (EMT), and the reduced expression of Claudin-1 in airway epithelial tissue is an important marker of EMT [5,6]. Claudin-1 is expressed in melanoma, a non-epithelial malignant tumor, and contributes to cell migration [7]. Similarly, claudin-1 expression promotes cell adhesion and migration in Langerhans cells and other cells [8]. In esophageal squamous cell carcinoma, claudin-1 is expressed in cancer cells and induces proliferation and migration of cancer cells through certain signaling pathways [9]. Then, does the expression and function of Claudin-1 change during the pathological process of asthma characterized by smooth muscle cell proliferation? Is Claudin-1 a protein expressed in both airway epithelial and smooth muscle cells and involved in airway remodeling in

asthma?. The coagulation system is activated in the presence of pulmonary inflammatory diseases. The basic mediator of coagulation is tissue factor, which initiates coagulation and activates thrombin [10]. Thrombin, a key protease in the clotting system, is involved in almost every stage of asthma development, including changes in airway epithelium and smooth muscle layer during airway remodeling. Previous studies have shown that thrombin has close relationship with TJs, thrombin induced rapid decomposition of claudin-5 from TJs of vascular endothelial cells [11]. In mouse brain microvascular endothelial cells, thrombin reduced expression of occludin, claudin-5 and zonula occludens-1 [12]. In canine renal epithelial cells (MDCK), thrombin can activate the endogenous Gα12 leading to the loss of claudin-1 and occludin to affect the integrity of TJ proteins family [13]. Based on the above research and the problems brought by it, we guess if thrombin affect the expression of Claudin-1 during the pathological process of airway remodeling in asthma, so we conducted the following experiments.

3. Materials and Methods

3.1. Reagents

The Chicken egg ovalbumin (OVA), aluminum hydroxide, hyperin, BAY 11-7082 and Rabbit-anti-mouse SMA were purchased from Sigma Aldrich, St. Louis, MO, USA. Bovine thrombin was purchased from Haematologic Technologies Inc. The bicinchoninic acid protein assay kit was procured from Pierce Biotechnology, Rockford, IL, USA. Mouse monoclonal anti-ASM, rabbit monoclonal anti-claudin-1 antibody were purchased from were purchased from Cell Signaling Technology, Danvers, MA, USA. DyLight488-conjugated goat anti-mouse IgG and DyLight594-conjugated goat anti-rabbit IgG were from Beijing Biosynthesis Biotechnology Co., Ltd (Beijing, China). RCR kits and Transfection Kit were purchased from GenePharma.

3.2. Animal and Sensitization

The BABL/C mice were purchased from Beijing Charles River Company. Sensitize the mouse with 1 mg OVA (s.c.) and 1 ml aluminum hydroxide (200 mg/ml, s.c.) on days 0 and 7. The control group mouse received an i.p. injection of normal saline on days 0 and 7 accordingly.

3.3. Inhalational Exposure

Aerosolize the sensitized mouse with OVA (20 mg/ml) for 30 min twice a day for 6 weeks, by using the AZWELL UN-511 ultrasonic nebulizer (Osaka, Japan), from day 14 (TIW) (OVA group). While the control group mice were exposed to aerosolized normal saline (NS) at the same time.

3.4. Inhalation of Thrombin

Treat the mice of thrombin + OVA group with aerosolized thrombin for 30 min for 4 weeks from day 14. Immunofluorescence. The expression of Claudin-1 in lung tissue of asthmatic model mice was detected by immunofluorescence. Paraffin slices were prepaired. Sections were sealed with 10% normal goat serum and incubated at room temperature for 30 min. Add two kinds of primary antibody diluted according to the instructions, and incubate at 37℃ for 1 hour. Rinse with PBS and add two kinds of fluorescent secondary antibodies according to the instructions, put them into a shading box to avoid light, and incubate at room temperature for 1 hour. DAPI staining: drop DAPI reagent on the tissue and avoid light for 10 minutes. Wash with PBS. The anti-fluorescence quenching agent was sealed and photographed under fluorescence microscope.

3.5. RT-PCR

Claudin-1 expression in lung tissues was detected by RT-PCR. Total RNAs, extracted from airway smooth muscle cells by Nucleospin RNAII kit, were reverse transcribed into cDNAs by reverse-transcription system. cDNAs were subjected to qRT-PCR analysis using ABsolute Blue QPCR SYBR green mix under conditions below: 94°C for 30 s, 40 cycles with 59°C for 30 s and 72°C for 30 s, 72°C for 10 min. Relative levels of genes were normalizedto GAPDH with the followingprimers:Claudin-1(Forward CTAGTTTCAGAAGTTTGGCCTGGTA, Reverse CTGCCAGCTTCCATTAGACTTTG) GAPDH(-Forward AATGACCCCTTCATTGAC, Reverse TCCACGACGTACTCAGCGC) SiRNA interfereation experiment The airway smooth muscle cells in logarithmic growth phase were collected. After the culture was stopped, the single-cell suspension made after digestion of the cells was collected. A sterile 6-well plate was seeded in the plate with 2.0×105 pieces per well and put back into an incubator at 37℃ for use. SiRNA sequences: Claudin-1(Forward 5 '- GGCAGAUACAGUGCAAAGUTT - 3', Reverse 5 '- ACUUUGCACUGUAUCUGCCTT - 3') NC (Forward 5 '- UUCUCCGAACGUGUCACGUTT - 3', Reverse 5 '- ACGUGACACGUUCGGAGAATT - 3') According to the instructions of the transfection kit, SiRNA10μl and Lipofectamine 3000 were diluted and mixed with 100μl serum-free culture solution. The culture medium in the 6-well plate was changed into fresh culture medium without serum. Mixed liquid was slowly dropped into the 6-well plate. After that, the 6-well plate was put back into the 37℃ incubator for further incubation for 4-6h. The culture medium was changed into a complete culture medium containing serum, and then the 6-well plate was put back into the 37℃ incubator for further culture. The RNA was detected by RT-PCR after being cultured for 24 hours.

3.6. Transwell Assay

Si-NC ASMC and si-Claudin 1ASMC were inoculated into 96-well plates with a density of 2×105 cells/mL. When the cells reached 60%, 0.4ul of the configured thrombin and 0.4ul of PBS were added into each well to stimulate ASM cells and siRNA-transfected ASM cells. Airway smooth muscle cells with different treatments were suspended with serum-free growth medium and plated in the upper

chamber of well coated with matrigel. Growth medium containing serum was added into the lower chamber. Following incubation for 24 h and removal of the membranes, the cells in the lower chamber were fixed and then stained with crystal violet. The number of cells was counted under microscope.

Cell proliferation detected by CCK8 Airway smooth muscle cells with different treatments were placed in an incubator at 37℃ for pre-culture for 36 hours; Add 10ul CCK solution to each well after 36 hours (be careful not to produce bubbles); Incubate in 37℃ incubator for 2 hours; After 2 hours, the culture plates were taken out and the absorbance was measured at 450nm on a microplate analyzer. Absorbance was recorded and cell viability was calculated in each group. Western Blot for claudin-1 p-p65 and p65 Mouse airway smooth muscle cells (2×104 cells/well) were seeded and incubated with thrombin(1u/ml), NF-κB inhibitor BAY 11-7082(5.0uM)and hyperin(50uM)for 48 h before the functional assays. Protein concentration of the lysates was determined by bicinchoninic acid protein assay kit. The lysates (40 μg) were subjected to electrophoretic separation and transferred to polyvinylidene difluoride membranes. The membrane was blocked and then incubated with primary antibodies against claudin-1(1:1000), p-p65(1:2000), p65(1:2000) and GAPDH (1:1000). After incubation with peroxidase-conjugated secondary antibodies, the membranes were subsequently conducted with ECL chemiluminescence detection kit and visualized by Odyssey Infrared Imaging System. The signals of bands in the membranes were quantified by Quantity One 4.6.2 software.

Statistics. The results of measurement data were expressed as mean±standard deviation. Independent sample T test was used for comparison between the two groups. At the same time, homogeneity test of variance has been carried out before the test and the condition of homogeneity of variance is satisfied. All data were analyzed using SPSS19 software. p values less than 0.05 were considered statistically significant.

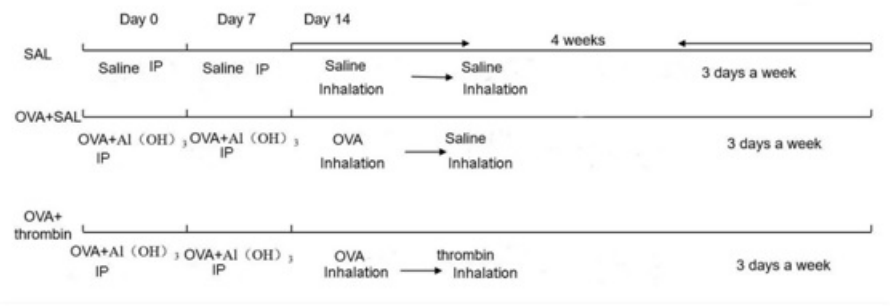

Figure 1. A time line showing the time when each treatment was administered.

4. Results

Effects of thrombin on the expression of Claudin-1 in lung tissue of OVA sensitized mice. The expression of Claudin-1 was evaluated by immunofluorescence. In figure 2, compared with normal mice, the expression of Claudin-1 in the epithelial cells of the lung tissue of OVA-sensitized mice was significantly reduced, and the expression of Claudin-1 was increased in ASM. The immunostaining area and intensity of Claudin-1 in ASM of the OVA + thrombin group were significantly higher than that of the OVA + Sal group, while the fluorescence intensity of Claudin-1 could hardly be detected between epithelial cells. These results showed that the expression of Claudin-1 migrated in the airway of asthmatic model mice, and this abnormal expression was made more pronounced by thrombin.

4.2. Expression of Claudin-1 mRNA in Lung Tissue of mice in Each Group.

RT-PCR was used to detect the expression of claudin-1 in the lung tissues of mice. In figure 3, there was no significant difference in the expression of claudin-1 in the lung tissues of mice in the asthma group and the normal control group ($P = 0.051$). Compared with asthmatic mice, the mRNA expression of Claudin-1 was increased in lung tissues of asthmatic mice after thrombin atomization ($P < 0.01$). Knocking down expression of claudin-1 reduced migration of airway smooth muscle cells Transwell detected the migration of two kinds of ASM cells, and the results showed that the migration ability of si-Claudin-1 ASMC was slightly decreased compared with that of si-NC ASMC, after thrombin stimulation, the migration of Si-Claudin-1 ASMC was significantly reduced compared with that of si-NC ASMC. Knocking down expression of claudin-1 reduced proliferation of airway smooth muscle cells CCK8 detected the proliferation of two kinds of ASM cells. The results showed that the proliferation ability of si-Claudin-1 ASMC was slightly decreased compared with that of si-NC ASMC, after thrombin stimulation, the proliferation of si-claudin-1 ASMC was significantly decreased compared with si-NC ASMC. Effects of NF-κB signaling pathway inhibitor on thrombin-promoted expression of Claudin-1 in smooth muscle cells Western blot was used to detect the expression level of Claudin-1 protein (Fig. 7). The results showed that thrombin could promote the increase of Claudin-1 expression in ASMCs, compared with the control group, $p<0.001$; and thrombin-stimulated ASMCs were treated with NF-κB after administration of NF-κB. After inhibitor intervention, the expression of Claudin-1 decreased, which was statistically significant compared with the thrombin group ($p = 0.024$).

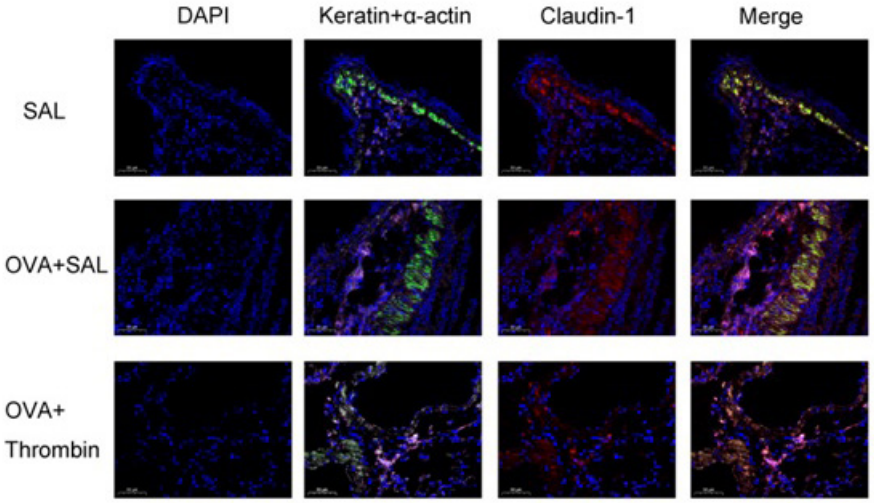

Figure 2. Expression of Claudin-1 in lung tissues of mice in each group was detected by immunofluorescence Green fluorescence represented epithelial cells, pink fluorescence represented smooth muscle cells, and red fluorescence represented Claudin-1. In SAL group, Claudin-1 was mainly expressed in epithelial cell layer, but not in smooth muscle layer. In OVA group, claudin-1 expression was decreased in the upper cortex and in the smooth muscle layer. The expression of Claudin-1 in OVA+ Haemobin group was significantly decreased in epithelial cells, but significantly increased in smooth muscle cells.

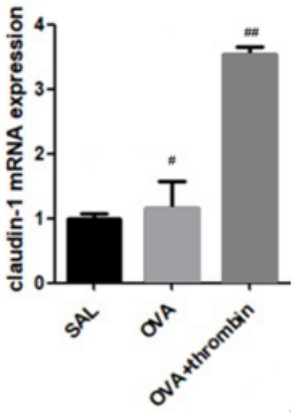

Figure 3: Expression of Claudin-1 in lung tissues of mice in each group was detected by RT-PCR

RT-PCR was used to detect the expression level of Claudin-1 gene in the lung tissues of mice in each group. As can be seen from the histogram, there was no statistical difference in the expression level of Claudin-1 mRNA in the lung tissues of asthmatic mice and normal control mice (# P =0.051). Compared with asthmatic mice, the mRNA expression of Claudin-1 in lung tissues of asthmatic mice after thrombin atomization was increased, and the comparison between the two groups was statistically significant (## P < 0.001).

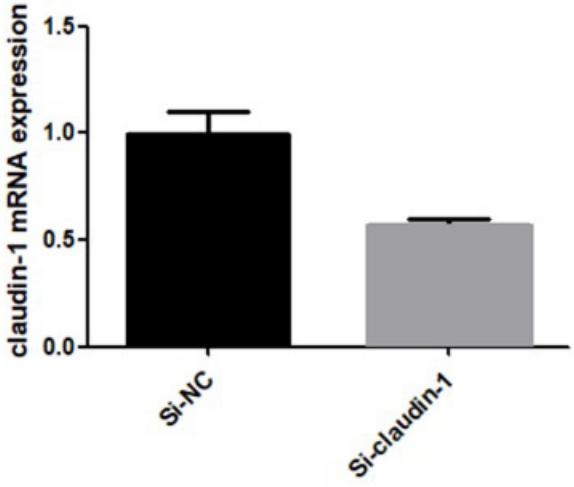

Figure 4: RT-PCR was used to detect whether the expression of claudin-1 was lower 24 hours after transfection, and the results showed that the expression of claudin-1 was lower after transfection, which was statistically significant compared with the control group (p <0.01).

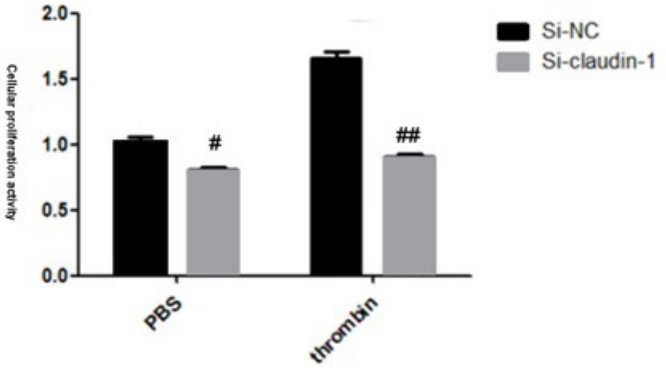

Figure 5: CCK8 detected the proliferation of two kinds of ASM cells, and the results showed that the proliferation of Claudin-1 siRNA ASMC was slightly decreased compared with NC siRNA ASMC (# p=0.01). After thrombin stimulation of both kinds of cells, the results showed that the proliferation of Claudin-1 siRNA ASMC was significantly decreased compared with NC siRNA ASMC (## p <0.01).

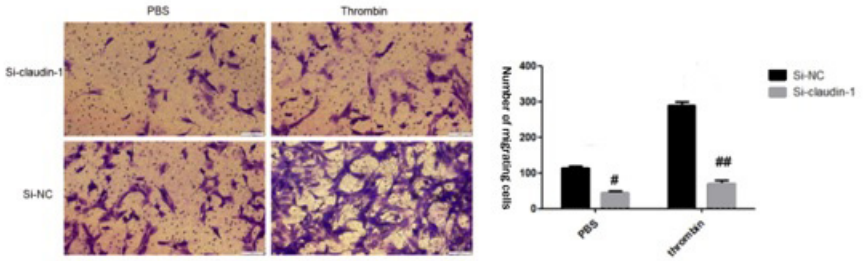

Figure 6: Transwell detected the migration of two types of ASM cells, and the results showed that the migration of Claudin-1 siRNA ASMC was slightly decreased compared with NC siRNA ASMC (#p<0.01). After thrombin stimulation of both types of cells, the results showed that the migration of Claudin-1 siRNA ASMC was significantly decreased compared with NC siRNA ASMC (## p<0.01).

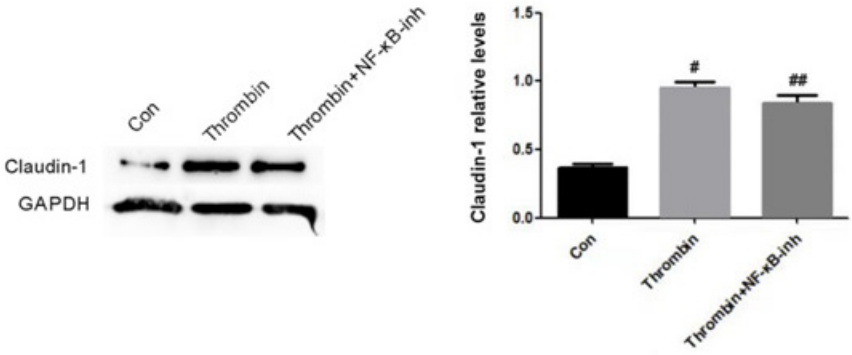

Figure 7: Effects of NF-κB signaling pathway inhibitors on claudin-1 expression Western blot was used to detect the effect of signaling pathway inhibitors on claudin-1 expression. Compared with the control group, thrombin increased claudin-1 expression (#p<0.001), but the addition of NF-κB inhibitor reduced the expression of Claudin-1 compared with thrombin group (##p =0.024).

4.3. Effects of Hyperin on Thrombin Promoting Claudin-1 Expression

Western blot was used to detect the expression of Claudin-1 (Figure. 8), and the results showed that compared with PBS group, thrombin up-regulated the expression of Claudin-1 in smooth muscle cells (p =0.008), while the expression of Claudin-1 in smooth muscle cells was down-regulated after hyperoside intervention in thrombin stimulated ASMC. Compared with thrombin group, it was statistically significant (p <0.001).

4.4. Effects of Hyperin on NF-κB Signaling Pathway Induced by Thrombin

Western blot was used to detect the expression level of phosphorylated p65, as shown in Figure. 9. Compared with PBS group, thrombin up-regulated the phosphorylation level of p65 in smooth muscle cells (p<0.001), after hyperin intervention, the phosphorylation level of P65 in thrombin stimulated ASMC decreased compared with that in thrombin group, and the comparison between the two groups was statistically significant (p <0.001).

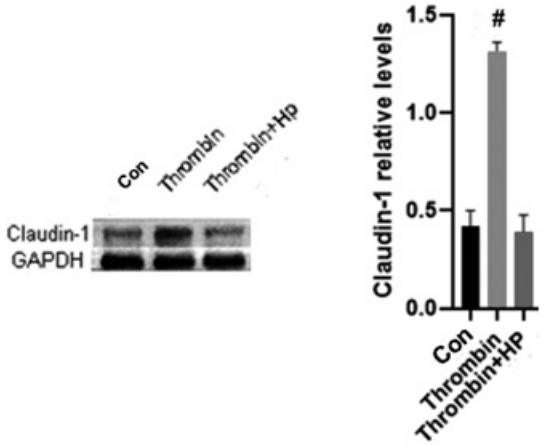

Figure 8: Expression of Claudin-1 in airway smooth muscle cells treated with different drugs Western blot was used to detect the expression of Claudin-1 in airway smooth muscle cells treated with different drugs. Thrombin could enhance the expression of Claudin-1, which was statistically significant compared with PBS group (# p =0.008), while hyperin intervention could decrease the expression of Claudin-1 in thrombin stimulated cells, which was statistically significant compared with thrombin group (# p=0.015).

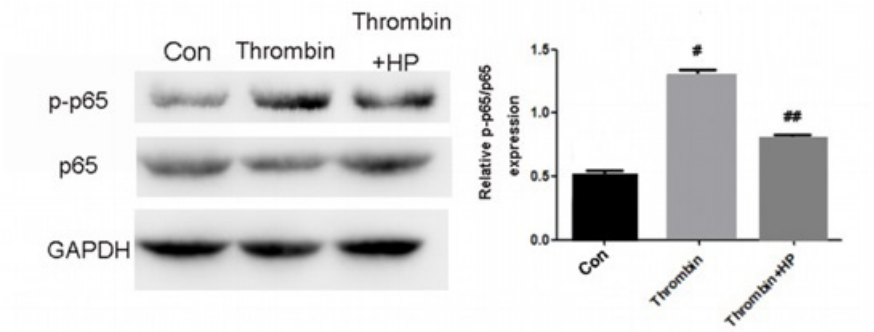

Figure 9: Effects of hyperin on p65 phosphorylation in thrombin treated airway smooth muscle cells Western blot was used to detect the inhibitory effect of hyperin on thrombin induced phosphorylation of P65. Thrombin increased the phosphorylation of p65 in ASMC (#p<0.001), and the phosphorylation level of p65 in ASMC stimulated by thrombin decreased after hyperin intervention, which was statistically significant compared with thrombin group (## p<0.001).

5. Discussion

Asthma is a major chronic disease which seriously threatens public health worldwide and is also one of the most common chronic diseases in children [14]. Inflammatory process leads to the change of the airway wall structures, these changes include goblet cell growth, bronchial mucus gland enlargement, epithelial cells peeling off, smooth muscle mass increases, or new vasculature angiogenesis, and many changes of extracellular matrix [15]. In this study, we mainly discussed the epithelium and smooth muscle. Through animal experiments and cell experiments, and combined with a large number of references, we proved that the biomarker Claudin-1, which is involved in both these two aspects, promotes the occurrence and development of asthma by participating in the destruction of epithelial structure and the proliferation of smooth muscle. Airway epithelial cells are the first continuous line of defense against damage caused by inhaled harmful substances, including pathogens, pollutants, and airborne allergens. It has been reported that bronchial epithelial cells of asthma patients have imperfect anti-microbial invasion due to structural damage and ciliary movement disorders [16]. In fact, airway epithelial damage is also associated with severe airway hyperresponsiveness (AHR) [17]. In addition, epithelial injury may lead to enhanced proinflammatory activity and secretion of growth factors [18], leading to airway remodeling, inflammation, and hyper responsiveness. These changes are closely related to the airway epithelial tight junction protein family. Transmembrane proteins of TJ include ligation adhesion molecules, closure proteins, and claudins, which are anchored to the cytoskeleton by zo-1, -2, -3, and cingulate proteins [19,20]. Previous studies have demonstrated that the expression of Claudin-1 is localized in the trachea and bronchioles through immunofluorescence localization detection [21]. Claudin-1 is closely related to the destruction of epithelial structure in respiratory diseases and plays a key role in the pathological process of asthma [22]. As shown in Figure 2, the expression of Claudin-1 in the airway epithelium of normal control mice was complete and continuous, while the expression of Claudin-1 in the airway epithelium of OVA-sensitized mice was reduced.

Once the barrier function of the airway epithelium is lost, the airways become vulnerable to inhaled environmental factors. Long-term exposure to these environmental factors will lead to chronic inflammatory environment and airway remodeling, which is mainly characterized by the deposition of extracellular matrix proteins and excessive secretion of mucus, thereby damaging the structure and

function of the lung [23]. In the experiment, we detected the cell proliferation of ASMC and Claudin-1 SiRNA-interfered ASMC respectively, and the results showed that the proliferation ability of the former was slightly higher than that of the latter. Thrombin was used to intervene ASMC and Claudin-1 SiRNA-interfered ASMC respectively, the results showed that the proliferation of claudin-1 SiRNA ASM cells was significantly lower than that of normal ASM cells after thrombin intervention. The same result was found in the experiment of Transwell. Claudin-1 gene knockout has been shown to reduce proliferation in keratinocytes [24]. Claudin-1 gene knockout has a negative effect on cell proliferation in endometrial cancer cells and gastric cancer cells [25]. These results are consistent with our results showing that claudin-1 is closely related to the proliferation of airway smooth muscle cells, and the low expression of claudin-1 can limit the proliferation of ASM cells promoted by thrombin. However, the specific mechanism is still unknown, but previous studies have shown that the promotion of cell proliferation by claudin-1 in keratinocytes may be related to p-Akt [26], and in endometrial cancer cells, claudin-1 expressed in the nucleus can promote cell migration and invasion through PI3K/Akt [27]. It also provides a direction for our further research. In humans and experimental animals, activation of the coagulation system also occurs in the respiratory tract, and recent studies have pointed out an important role in the pathogenesis of allergic inflammation and airway remodeling in asthma [28,29]. The source of pulmonary clotting factors may be plasma proteins leaking from inflammatory blood vessels or proteins synthesized and secreted by lung epithelial cells [30,31]. Physiological level of thrombin production in airway may play a role in mucosal hemostasis, fibrin-mediated bacterial inhibition, or enhancement of defense mechanism [32]. However, pathological conditions, such as mucosal hypersensitivity and inflammation, excessive thrombin production and fibrin deposition, cause harmful effects on the airway and cover its physiological regulatory mechanism [33-35]. Claudin-1 expression in airway epithelial cells in asthma was reduced, and the decrease was more obvious after thrombin intervention, but from RT-PCR test we found that the expression of claudin-1 in lung tissue in asthmatic mice was not decreased compaired with nomal mice. Thrombin intervention of asthma model made claudin-1 expression in lung tissue significantly enhanced. These results proved that the expression of claudin-1 in epithelial cells reduced, and at the same time, there should be an enhanced expression of claudin-1 in other kinds of cells in lung tissue. Combined with our immunofluorescence result, claudin-1 expressed in airway smooth muscle cells was increased, and the degree of increase was greater than that of decrease in epithelial cells. In fact, previous studies on the mechanism of pulmonary arterial hypertension have confirmed that Claudin-1 is involved in the pathogenesis of pulmonary arterial hypertension (PAH). Claudin-1 can be expressed in vascular smooth muscle cells to promote cell proliferation and migration. NF-κB inhibitor BAY 11-7082 inhibited the upregulation of Claudin-1 [36]. Claudin-1 expression in the nucleus has been shown to be mediated by NF-κB in cancer studies [37,-39]. In our study, thrombinase-treated airway smooth muscle cells were treated with a NF-κB inhibitor (BAY 11-7082), which showed that BAY 11-7082 inhibited thrombinase-induced up-regulation of Claudin-1.

Hyperin, a flavonoid, has been widely used in traditional Chinese medicine due to its antioxidant [38], anti-inflammatory [39], anti-tumor [40] and other properties. Studies on its therapeutic effects include inhibition of lipopolysaccharide induced acute liver injury in mice (Huang, C., et al., 2015), inhibition of lipopolysaccharide induced nitrite production in rat peritoneal macrophages [41], regulation of adhesion of extracellular matrix in cancer progression [42], and inhibition of pseudomonas aeruginosa biofilm formation [43]. It has been proved that hyperin can inhibit the production and activity of thrombin in the mechanism of coagulation [44]. However, there are few studies on the role of hyperin in the treatment of asthma. Some studies have confirmed that hyperin can inhibit the accumulation of inflammatory cells and the levels of IL-4, IL-5, IL-13 and IgE in OVA-induced asthmatic model mice through Nrf2 signaling pathway [45], so its role in asthma has attracted more and more attention. It is well known that TGF-β1 is a key regulator of the fibrotic response, leading to abnormal airway smooth muscle cell function during asthma [46-50]. TGF-β1 is elevated in the airway of asthma and promotes theproliferation and migration of airway smooth muscle cells [50-52]. TGF- β1 also promotes airway remodeling by enhancing the extracellular matrix such as fibronectin, tendinin and collagen [53,54]. Therefore, TGF-β1 induction of airway smooth muscle cells has been widely used in in vitro models of asthma [55]. In our previous study, TGF-β1 was used to intervene airway smooth muscle cells and hyperin was treated with different concentrations to confirm the effect of hyperin and determine the appropriate drug concentration [55-71]. Hyperin reduced the phosphorylation of P65, thereby decreasing TGF-β 1-induced NF-κB activity in a dose-dependent manner. After demonstrating the role of hyperin in an in vitro model of asthma, we used hyperin to intervene in thrombinase-treated airway smooth muscle cells and found that the increase in thrombinase-induced claudin-1 expression could be inhibited by hyperin. We also examined NF-κB activation of airway smooth muscle cells in the thrombin group and the thrombin + hyperin group. The results showed that hyperin did inhibit the increased phosphorylation of p65 induced by thrombin, resulting in the decreased expression of Claudin-1. In summary, regulation of claudin-1 expression in airway smooth muscle cells is an important mechanism of thrombin promoting the proliferation of smooth muscle cells. Thrombin can increase the expression of Claudin-1 in airway smooth muscle cells of asthmatic mice through NF-κB signaling pathway. Hyperin inhibits the expression of Claudin-1 in thrombin - induced airway smooth muscle cells. These experiments provide new ideas for finding molecular markers and therapeutic targets of asthma in clinic.

References

1. Ahdieh M, Vandenbos T, Youakim A. Lung epithelial barrier function and wound healing are decreased by IL-4 and IL-13 and enhanced by IFN-γ. American Journal of Physiology - Cell Physiology. 2001; 281(650-6): C2029-C2038.
2. Agostinis, P., Vantieghem, A, Merlevede W. Hypericin in cancer treatment: more light on the way. The International Journal of Biochemistry & Cell Biology. 2002; 34: 221-241.
3. Anastasia Karioti, Anna Rita Bilia. Hypericins as potential leads for new therapeutics. Int J Mol Sci. 2010; 11(2): 562-94.
4. Barbato A, Turato G, Baraldo S. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med. 2006; 174: 975-981.
5. Chen, G. and Khalil, N. TGF-β1 increases proliferation of airway smooth muscle cells by phosphorylation of map kinases. Respiratory Research. 2006; 7: 2.
6. Chen M, Shi JT, Lv ZQ. Triptolide inhibits TGF-β1 induced proliferation and migration of rat airway smooth muscle cells by suppressing NF-ϰB but not ERK1/2. Immunology. 2014; 144: 486-494.
7. Cheng W, Yan K, Xie LY. MiR-143-3p controls TGF-β1-induced cell proliferation and extracellular matrix production in airway smooth muscle via negative regulation of the nuclear factor of activated T cells 1. Molecular Immunology. 2016; 78: 133-139.
8. Coyne CB, Gambling TM, Boucher RC. Role of claudin interactions in airway tight junctional. Am J Physiol Lung Cell Mol Physiol. 2003; 285(5): L1166-1178.
9. De Boer JD, Majoor CJ, van't Veer C. Asthma and coagulation. Blood. 2012; 119: 3236-3244.
10. Dhawan P, Singh AB, Deane NG. Claudin-1 regulates cellular transformation and metastatic behavior in colon cancer. J Clin Invest. 2005; 115: 1765-1776.
11. Enrique Archiniegas, Carmen Yudith Neves, Daniel Candelle. Thrombin and its protease-activated receptor-1 (PAR1) participate in the endothelial-mesenchymal transdifferentiation process. DNA Cell Biol. 2004; 23(12): 815-25.
12. Ernesto Sabath, Hideyuki Negoro, Sarah Beaudry. Gα12 regulates protein interactions within the MDCK cell tight junction and inhibits tight-junction assembly. J Cell Sci. 2008; 121(Pt 6): 814-824.
13. Firszt R, Francisco D, Church TD. Interleukin-13 induces collagen type-1 expression through matrix metalloproteinase-2 and transforming growth factor-beta1 in airway fibroblasts in asthma. Eur Respir J. 2014; 43(2): 464-473.
14. Fischer KD, Hall SC, Agrawal DK. Vitamin D supplementation reduces induction of epithelial-mesenchymal transition in allergen sensitized and challenged mice. PLoS One. 2016; 11(2): e0149180.
15. Fischer A, Gluth M, Pape UF. Adalimumab prevents barrier dysfunction and antagonizes distinct effects of TNF-alpha on tight junction proteins and signaling pathways in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2013; 304(11): G970-979.
16. Fortier AM, Asselin E, Cadrin M. Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through claudin1 up-regulation. J Biol Chem. 2013; 288(16): 11555-11571.
17. Gon Y, Hashimoto S. Role of airway epithelial barrier dysfunction in pathogenesis of asthma. Allergol Int. 2018; 67(1): 12-17.
18. Hackett TL, Singhera GK, Shaheen F. Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to RSV and air pollution. Am J Respir Cell Mol Biol. 2011; 45: 1090-1100.
19. Heijink IH, Brandenburg SM, Noordhoek JA. Characterisation of cell adhesion in airway epithelial cell types using electric cell-substrate impedance sensing. Eur Respir J. 2010; 35: 894-903.
20. Huang C, Yang Y, Li WX. Hyperin attenuates inflammation by activating PPAR-γ in mice with acute liver injury (ALI) and LPS-induced RAW264.7 cells. International Immunopharmacology. 2015; 29: 440-447.
21. Huang J, Zhang L, He C. Claudin-1 enhances tumor proliferation and metastasis by regulating cell anoikis in gastric cancer. Oncotarget. 2015; 6(3): 1652-65.
22. Hung CH, Wang CC, Suen JL, Kuo. A research: altered pattern of monocyte differentiation and monocyte-derived TGF-beta1 in severe asthma. Sci. Rep. 2018; 8: 919.
23. Jeroen Bastiaans, Jan C, van Meurs, Conny van Holten-Neelen. Thrombin induces epithelial-mesenchymal transition and collagen production by retinal pigment epithelial cells via autocrine PDGF-receptor signaling. Invest Ophthalmol Vis Sci. 2013; 54(13): 8306-14.
24. Jeong CH, Seok IS, Petriello MC. Arsenic downregulates tight junction claudin proteins through p38 and NF- k B in intestinal epithelial cell line, HT-29. Toxicology. 2017; 379: 31-39.
25. Jian Wu, FengXia Gao, Tao Xu. CLDN1 induces autophagy to promote proliferation and metastasis of esophageal squamous carcinoma through AMPK/STAT1/ULK1 signaling. J Cell Physiol. 2020; 235(3): 2245-2259.
26. Kalita M, Tian B, Gao B. Systems approaches to modeling chronic mucosal inflammation. Biomed Res Int. 2013: 505864.
27. Kawanami D, Matoba K, Kanazawa Y. Thrombin induces MCP-1 expression through Rho-kinase and subsequent p38MAPK/NF-jB signaling pathway activation in vascular endothelial cells. Biochem Biophys Res Commun. 2011; 411(4): 798-803.
28. Kim HJ, Lee SH, Jeong S. Protease-Activated Receptors 2-Antagonist Suppresses Asthma by Inhibiting Reactive Oxygen Species-Thymic Stromal Lymphopoietin Inflammation and Epithelial Tight Junction Degradation. Allergy Asthma Immunol. Res. 2019; 11(4): 560-571.
29. Ku SK, Kwak S, Kwon OJ. Hyperoside inhibits high-glucose-induced vascular inflammation in vitro and in vivo. Inflammation. 2014; 37: 1389-400.
30. Ku SK, Zhou W, Lee W. Anti-inflammatory effects of hyperoside in human endothelial cells and in mice. Inflammation. 2015; 38: 784-99.
31. Laitinen LA, Heino M, Laitinen A. Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis. 1985; 131: 599-606.

32. Lee JH, Won S, Stein DG. Progesterone attenuates thrombin-induced endothelial barrier disruption in the brain endothelial cell line bEnd.3: The role of tight junction proteins and the endothelial protein C receptor. Brain Res. 2015; 1613 :73-80.

33. Lee S, Park HS, Notsu Y. Effects of hyperin, isoquercitrin and quercetin on lipopolysaccharide-induced nitrite production in rat peritoneal macrophages. Phytotherapy Research. 2008; 22: 1552-1556.

34. Leotlela PD, Wade MS, Duray PH. Claudin-1 overexpression in melanoma is regulated by PKC and contributes to melanoma cell motility. Oncogene. 2007; 26: 3846-3856.

35. Levi M, Van der Poll T. Inflammation and coagulation. Crit Care Med. 2010; 38(2 suppl): S26-S34.

36. Martin TA, Jiang WG. Loss of tight junction barrier function and its role in cancer metastasis. Biochim Biophys Acta Biomembr. 2009; 1788(4): 872-891.

37. Matter K, Aijaz S, Tsapara A, Balda MS. Mammalian tight junctions in the regulation of epithelial differentiation and proliferation. Curr Opin Cell Biol. 2005; 17: 453-458.

38. Mauad T, Bel EH, Sterk PJ. Asthma therapy and airway remodeling. J Allergy Clin Immunol. 2007, 120: 997-1009.

39. Mirzapoiazova T, Kolosova IA, Romer L. The role of caldesmon in the regulation of endothelial cytoskeleton and migration. J Cell Physiol. 2005; 203(3): 520-528.

40. Morin PJ. Claudin proteins in human cancer: promising new targets for diagnosisand therapy. Cancer Res. 2005; 65: 9603-9606.

41. Nan Zhang, Tingting Zhao, Meirong Bi. Hyperin Ameliorates Proliferation, Migration, and Extracellular Matrix Formation in Airway Smooth Muscle Cells by Inhibiting Transforming Growth Factor-β1-Induced Nuclear Factor-κB Activation. Current Topics in Nutraceutical Research. 2021; 19: 222-227.

42. Nawijn MC, Hackett TL, Postma DS. E-cadherin: gatekeeper of airway mucosa and allergic sensitization. Trends Immunol. 2011; 32: 248-255.

43. Nobuyuki Kondo, Minetaro Ogawa, Hiromi Wada, Shin-Ichi Nishikawa. Thrombin induces rapid disassembly of claudin-5 from the tight junction of endothelial cells. Exp Cell Res 2009; 315(17): 2879-2887.

44. Peng Ye, Xi-Liang Yang, Xing Chen. Hyperoside attenuates OVA-induced allergic airway inflammation by activating Nrf2. Int Immunopharmacol. 2017; 44: 168-173.

45. Perrio MJ, Ewen D, Trevethick MA. Fibrin formation by wounded bronchial epithelial cell layers in vitro is essential for normal epithelial repair and independent of plasma proteins. Clin Exp Allergy. 2007; 37: 1688-1700.

46. Priyanka Chowdhury, Payel Dey, Sourav Ghosh. Reduction of metastatic potential by inhibiting EGFR/Akt/p38/ERK signaling pathway and epithelial-mesenchymal transition after carbon ion exposure is potentiated by PARP-1 inhibition in nonsmall-cell lung cancer. BMC Cancer. 2019; 19(1): 829.

47. Pouran Karimi, Nadereh Rashtchizadeh. Oxidative Versus Thrombotic Stimulation of Platelets Differentially activates Signalling Pathways. J Cardiovasc Thorac Res. 2013; 5(2): 61-65.

48. Puddicombe SM, Torres-Lozano C, Richter A. Increased expression of p21(waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. Am J Respir Cell Mol Biol. 2003; 28: 61-68.

49. Quante T, Ng YC, Ramsay EE. Corticosteroids reduce IL-6 in ASM cells via up-regulation of MKP-1. Am J RespirCell Mol Biol. 2008; 39: 208-217.

50. Raymond Kwan, Kok Sun Looi, M Bishr Omary. Absence of keratins 8 and 18 in rodent epithelial cell lines associates with keratin gene mutation and DNA methylation: Cell line selective effects on cell invasion. Exp Cell Res. 2015; 335(1): 12-22.

51. Robert J. Homer, Jack A. Elias. Airway remodeling in asthma: therapeutic implications of mechanisms. Physiology (Bethesda). 2005; 20: 28-35.

52. Royce SG, Tan L, Koek AA. Effect of extracellular matrix composition on airway epithelial cell and fibroblast structure: implications for airway remodeling in asthma. Ann Allergy Asthma Immunol. 2009; 102(3): 238-246.

53. Scotton CJ, Krupiczojc MA, Konigshoff M. Increased local expression of coagulation factor X contributes to the fifibrotic response in human and murine lung injury. J Clin Invest. 2009; 119: 2550-2563.

54. Sae-Kwang Ku, Tae Hoon Kim, Jong-Sup Bae. Anticoagulant activities of persicarin and isorhamnetin. Vascul Pharmacol. 2013; 58(4): 272-9.

55. Shimizu S, Gabazza EC, Ogawa T. Role of thrombin in chronicrhinosinusitis-associated tissue remodeling. Am J Rhinol Allergy. 2011; 25: 7-11.

56. Shinagawa K, Martin JA, Ploplis VA. Coagulation factor Xa modulates airway remodeling in a murine model of asthma. Am J Respir Crit Care Med. 2007; 175: 136-143.

57. T Holgate. Epithelium dysfunction in asthma. J. Allergy Clin. Immunol. 2007; 120(6): 1233-44.

58. Terada M, Kelly EA, Jarjour NN. Increased thrombin activity after allergen challenge: a potential link to airway remodeling? Am J Respir Crit Care Med. 2004; 169: 373-377.

59. Thevenot PT, Saravia J, Jin N. A research: radical-containing ultrafine particulate matter initiates epithelial-to-mesenchymal transitions in airway epithelial cells. Am. J. Respir. Cell Mol. Biol. 2013; 48: 188-197.

60. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat RevMol Cell Biol. 2001; 2: 285-293.

61. Tunggal JA, Helfrich I, Schmitz A. E-cadherin is essential for in vivo epidermal barrier function by regulating tight junctions. EMBO J. 2005; 24: 1146-1156.

62. Volksdorf T, Heilmann J, Eming SA. Tight Junction Proteins Claudin-1 and Occludin Are Important for Cutaneous Wound Healing. Am J Pathol. 2017; 187(6): 1301-1312.

63. Wagers SS, Norton RJ, Rinaldi LM. Extravascular fifibrin, plasminogen activator, plasminogen activator inhibitors, and airway hyperresponsiveness. J Clin Invest. 2004; 114: 104-111.

64. Wark PA, Johnston SL, Bucchieri F. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med. 2005; 201: 937-947.

65. Xiandong Cheng, Yi Wang, Huilong Chen. Claudin-1 regulates pulmo-

nary artery smooth muscle cell proliferation through the activation of ERK1/2. Biomed Pharmacother. 2017; 89: 983-990.

66. Xiao-Xiao Zuo, Ya Yang, Yue Zhang. Platelets promote breast cancer cell MCF-7 metastasis by direct interaction: surface integrin α2β1-contacting-mediated activation of Wnt-β-catenin pathway. Cell Commun Signa. 2019; 17(1): 142.
67. Xiaowen Qiu, Zhuo Lei,Zeyou Wang. Knockdown of LncRNA RHPN1-AS1 Inhibits Cell Migration, Invasion and Proliferation in Head and Neck Squamous Cell Carcinoma. J Cancer. 2019; 10(17): 4000-4008.
68. Yang FQ, Liu M, Li W. Combination of quercetin and hyperoside inhibits prostate cancer cell growth and metastasis via regulation of microRNA21. Mol Med Rep. 2015; 11: 1085-92.
69. Yingxin Zhao, Bing Tian, Rovshan G Sadygov. Integrative proteomic analysis reveals reprograming tumor necrosis factor signaling in epithelial mesenchymal transition. J Proteomic. 2016; 148: 126-38.
70. Yixuan Sun, Fengjun Sun, Wei Feng. Hyperoside inhibits biofilm formation of Pseudomonas aeruginosa. Exp Ther Med. 2017; 14(2): 1647-1652.
71. Y Zhou, GF Wang, L Yang. 1,25-Dihydroxyvitamin D3 prevented allergic asthma in a rat model by suppressing the expression of inducible nitric oxide synthase. Braz J Med Biol Res. 2015; 48(7): 654-664.