

## Increased Expression of Notch3 in Lung Tissue of Pulmonary Hypertension Mice Induced by Cigarette Smoke

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### Abstract

Pulmonary Hypertension (PAH) mice model was established by cigarette smoking method. C68B7J mice were randomly divided into control group and model group, 10 mice in each group. The control group did not do any treatment. The model group was treated with cigarette twice a day, 2 hours, 10/h, 6 days/week for 6 months. The Right Ventricular Pressure (RVSP) of mice was measured by BIOPAC Systems (MP150). The pulmonary arterioles were stained with collagen fibers (Van-Gieson method) and analyzed by Image Pro Plus software. The thickness of the middle layer of pulmonary arterioles and the diameter of pulmonary arterioles were measured, and the thickness of pulmonary arterioles was calculated. The expression of Notch3 protein in lung tissue was detected by Western Blot method. The results showed that: 1. Compared with the control group, the right ventricular pressure of the model group increased significantly, the pulmonary arterioles became thicker, the lumen became narrower, and the ratio of right ventricle to body weight increased significantly; 2. Compared with the control group, the expression of Notch3 protein in the lung tissue of the model group increased significantly.

**Keywords:** *Pulmonary hypertension; Smoking; Notch3*

### Introduction

Pulmonary hypertension is a kind of refractory disease characterized by pulmonary vasoconstriction and vascular remodeling. Its 5-year survival rate is only 25% [1]. Its etiology and pathological process have been paid great attention to, but it is still not fully elucidated. Many clinical and experimental data confirm that smoking is one of the main etiologies of many lung

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diseases, cardio cerebrovascular diseases, and pulmonary malignant tumors. Cigarette smoke and particulate matter contain many harmful components [2]. These harmful substances enter the human lung in the form of smoke, destroy the balance of oxidation/antioxidation [3], lead to lung injury such as chronic bronchitis [4], emphysema, and eventually lead to increased pulmonary artery pressure and even death [5]. Notch3 is an important factor closely related to hypoxia *in vivo*. Transcription factor [6,7]. Studies have shown that Notch3 expression is positively correlated with the degree of pulmonary inflammation and may be involved in the development of pulmonary hypertension. However, the role and expression of Notch3 in pulmonary diseases such as pulmonary hypertension remain unclear. In this study, a mouse model of pulmonary hypertension was established by cigarette smoke and observed. To observe the changes of Notch3 expression in the course of pulmonary hypertension, we hope to help understand the role of Notch3 in the occurrence and development of pulmonary hypertension.

## **Materials and Methods**

### **Materials and reagents**

Male C68B7J mice: 6-8 weeks, 21-26 g, all provided by Hunan Provincial Laboratory Animal Center. Animals have been certified by the Institute of Animal Administration (AAALAC, International Association for the Evaluation and Certification of Laboratory Animal Management). This experiment abides by the ethical principles of laboratory animal welfare. Animal processing procedures have been certified by the ethical committee of the First Clinical Medical College of Hunan Normal University. Notch3 and beta-Tubulin antibodies are purchased (From abcam).

### **Model preparation and experimental method**

1. C68B7J mice were randomly divided into the control group and model group with 10 mice in each group. The mice in model group were placed in a self-made smoking box for passive smoking (cigarette: Furong, produced by Hunan Zhongyan Industry Co., Ltd.). The cigarettes were treated twice a day, once in the morning and once in the afternoon, for 2 hours (5-10 minutes per hour), 3-4 hours between the afternoon and the afternoon, 10/h, 6 days/week for 6 consecutive days/month. Normal control group mice were not treated. During the experiment, the weight changes of mice were recorded weekly.
2. The right ventricular pressure (RVSP) of anesthetized mice was measured by the right ventricular pressure tester (BIOPAC Systems, MP150).
3. After killing mice, the right ventricle was cut off with scissors, then the right ventricle was cut down along the atrioventricular septum, weighed and the ratio of right ventricular mass to body mass was calculated.
4. Histopathological observation and morphological changes in pulmonary arteries were observed. Pulmonary tissues were fixed with 4% paraformaldehyde and 4% sucrose solution for 24 hours. After PBS was washed, paraffin was embedded and sections were stained with HE. Collagen fibers were stained with Van-Gieson method for pulmonary arterioles with diameters less than 50-100  $\mu\text{m}$ . Image Pro Plus software was used to analyze the images. The methods were as follows: According to the formula  $\text{MT\%} = \text{MT/ED} \times 100\%$  is used to calculate the percentage of the middle thickness of pulmonary arterioles to the lumen diameter, which reflects the change of pulmonary arterioles thickness and calculates the mean.

5. The expression of Notch3 in lung tissue of mice was washed with PBS at 4°C for 3 times. Each sample was added with 1 mL prepared cell lysate and lysed on ice for 30 minutes. The lysate products were collected. The supernatant was centrifuged at 4°C for 15 minutes at 12000 rpm and frozen at -80°C. The content of Notch3 protein in lung tissue was detected by Western-blot.

### Statistical analysis

All the data were expressed by  $\bar{x} \pm s$ . The student-t analysis method was used to compare the mean of control group and model group. The difference was statistically significant by  $p < 0.05$ .

## Results

### 1. Changes of the right ventricular systolic pressure (RVSP)

After 6 months of smoking, the RVSP of control group mice was  $(6.78 \pm 0.11)$  mm Hg, and the RVSP of smoking group mice was significantly higher than that of control group mice  $(11.22 \pm 0.29)$  mm Hg,  $p < 0.01$  (**FIG. 1**).

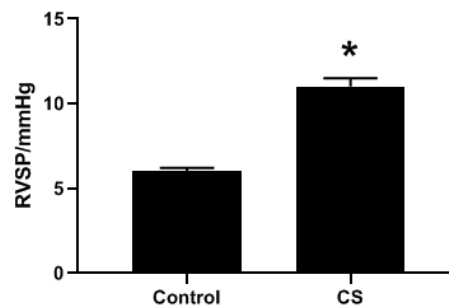


FIG. 1. Changes of right ventricular pressure in mice after 6 months of smoking. \* $p < 0.01$  VS control group.

### 2. Changes of pulmonary histopathology and pulmonary artery thickness in smoked mice

Compared with the normal mice, the pulmonary histopathological results showed that the pulmonary arterioles in the model group were significantly thicker and narrower (**FIG. 2**), indicating the occurrence of vascular remodeling. The results of VG staining showed that MT% in smoked mice was  $25.40 (\pm 0.69)$ , significantly higher than that in control mice,  $8.16 (\pm 0.12)$ ,  $p < 0.01$  (**FIG. 3**), suggesting that the pulmonary arterioles had changed.

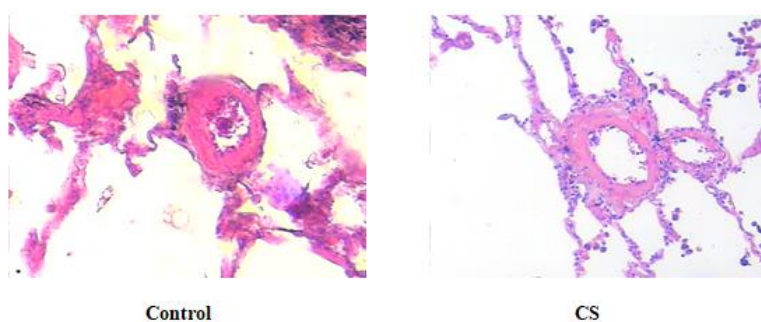


FIG. 2. HE staining of lung tissue, HE X 400.

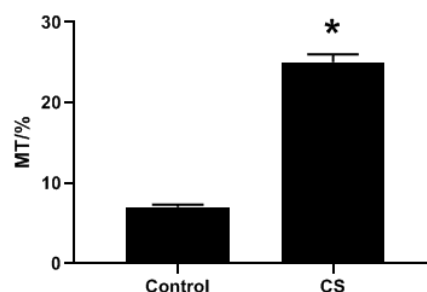


FIG. 3. Changes of pulmonary artery medial.

### 3. Changes of right ventricular hypertrophy

After 6 months of smoking, the ratio of right ventricle to body mass in smoked mice was 0.000 79 (+0.00002), which was significantly higher than that in control mice (0.000 58 (+0.000 02)),  $p < 0.01$  (FIG. 4).

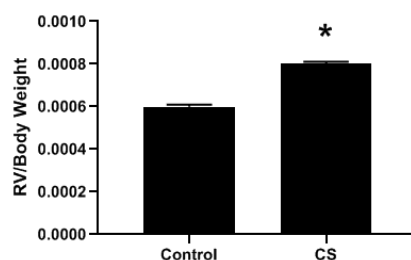


FIG. 4. Comparison of right ventricular hypertrophy in two groups of mice. \* $p < 0.01$  VS control group.

### 4. The expression of Notch3 in lung tissue of mice

In the control group, Notch3 protein expression was very low, while in the PAH model group, Notch3 protein level was significantly increased (FIG. 5), suggesting that long-term smoking may cause a relatively hypoxic environment in the lungs of mice and promote Notch3 expression.

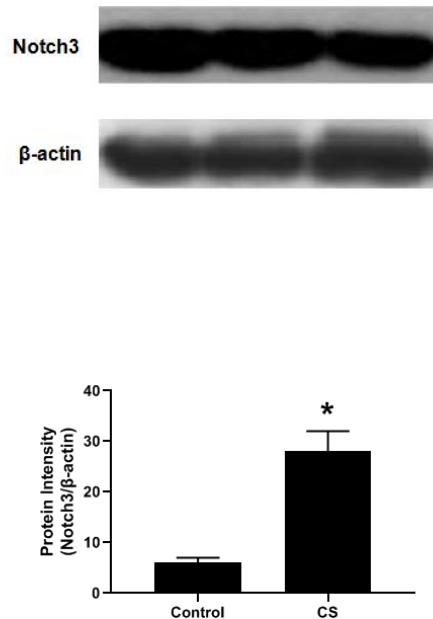


FIG. 5. Western-blot detection of Notch3 expression in lung tissues of each group. \* $p < 0.01$  VS control group.

## Discussion and Conclusion

Epidemiological investigation shows that pulmonary hypertension (PAH) is very common in the clinic and has a high degree of malignancy. The incidence of PAH in the population is second only to hypertension and coronary heart disease, and the 5-year survival rate is only 25%. Vasoconstriction and vascular remodeling are the main pathological basis of pulmonary hypertension: vasoconstriction is a functional change, which leads to elevated blood pressure; and vascular remodeling is the group. The change of weave quality will eventually lead to right ventricular hypertrophy and even pulmonary failure [8].

Studies have shown that the occurrence of PAH is closely related to smoking. Long-term inhalation of various harmful components and dust particles in tobacco can lead to lung injuries such as chronic bronchitis and emphysema and ultimately lead to increased pulmonary artery pressure and even death [9]. In view of the correlation between smoking and PAH, the establishment of smoke-related PAH animal model is to study the occurrence and development of PAH. An important approach to the process.

In this study, the PAH model was established by long-term smoking in mice. The results showed that the right ventricular pressure, pulmonary artery wall and right ventricular hypertrophy in mice were significantly increased after 6 months of smoking compared with the control group, which indicated that the PAH model induced by smoking was successful.

Notch3 is an important transcription factor related to hypoxia *in vivo*. It widely exists in mammals and humans. It is one of the most popular molecules in the study of hypoxic diseases [10-12]. Studies have shown that the expression of Notch3 is positively correlated with the degree of inflammation in lung tissues, and may be involved in airway remodeling and pulmonary hypertension [13]. However, for Notch3 and pulmonary diseases, the expression of Notch3 is positively correlated with the degree of inflammation in lung tissues. The relationship between Notch3 and chronic inflammatory diseases such as

pulmonary hypertension is still unclear. In this study, the expression of Notch 3 was detected in the pulmonary tissues of the mice with pulmonary hypertension. The results showed that the expression of Notch 3 protein was very low in the control group but significantly increased in the PAH model group, suggesting that Notch 3 plays an important role in chronic inflammatory diseases such as pulmonary hypertension.

After long-term smoking, Notch3 increased significantly in mice. It is speculated that Notch3 played an important role in the development of pulmonary hypertension caused by smoking. On the one hand, long-term smoking may cause a relatively hypoxic environment in the lungs, resulting in abnormal increase of Notch3 expression, resulting in increased vascular wall thickness and pressure; on the other hand, long-term smoking may seriously damage the body. The antioxidant system, but the specific role of Notch3 pathway in the progression of pulmonary hypertension remains to be further studied.

### Conflicts of Interest

The authors declare that they have no conflicts of interest with the contents of this article.

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