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Activation of the AMPK/Nrf2 pathway ameliorates LPS-induced acute lung injury by inhibiting oxidative stress and reducing inflammation

Haoxuan Li[1], Yiting Nie[1†], Hongyu Hui[1†], Xinxin Jiang[2†], Yuanyuan Xie[1†] and Cong Fu[1*]

Abstract

Background Numerous diseases-related acute lung injury (ALI) contributed to high mortality. Currently, the therapeutic effect of ALI was still poor. The detailed mechanism of ALI remained elusive and this study aimed to elucidate the mechanism of ALI.

Method This study was performed to expose the molecular mechanisms of AMPK/Nrf2 pathway regulating oxidative stress in LPS-induced AMI mice. The mouse ALI model was established via intraperitoneal injection of LPS, then the lung tissue and blood samples were obtained, followed by injection with Dimethyl fumarate (DMF). Finally, Western blot, HE staining, injury score, lung wet/dry ratio, reactive oxygen species (ROS) and ELISA were used to elucidate the mechanism of AMPK/Nrf2 pathway in LPS -induced acute lung injury by mediating oxidative stress.

Results The lung tissue injury score was evaluated, showing higher scores in the model group compared to the AMPK activator and control groups. DCFH-DA indicated that LPS increased ROS production, while AMPK activator DMF reduced it, with the model group exhibiting higher ROS levels than the control and AMPK activator groups. The lung wet/dry ratio was also higher in the model group. Western blot analysis revealed LPS reduced AMPK and Nrf2 protein levels, but DMF reversed this effect. ELISA results showed elevated IL-6 and IL-1β levels in the model group compared to the AMPK activator and control groups.**Conclusion**:

Conclusion Activating the AMPK/Nrf2 pathway can improve LPS-induced acute lung injury by down-regulation of the oxidative stress and corresponding inflammatory factor level.

Keywords Acute lung injury, AMPK/Nrf2, Oxidative stress, ALI

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Introduction

Acute lung injury (ALI) is a significant and prevalent lung disease that poses a substantial burden on healthcare systems globally. It is associated with high morbidity and mortality rates, making it a critical concern for healthcare professionals. ALI is characterized by acute respiratory insufficiency, which manifests as impaired gas exchange and inadequate oxygenation of the blood. This respiratory insufficiency leads to symptoms such as tachypnea, which is characterized by rapid and shallow breathing. The increased respiratory rate is an adaptive response to compensate for the impaired gas exchange [1, 2]. Although numerous treatment methods such as respiratory support therapy, surgery and drug treatment have been used to treat the patients with ALI, nearly 40 ~ 50% of patients with ALI suffer from the death all across the world [3, 4]. Therefore, effective strategies for improving therapeutic effect and prognosis of patients with ALI are urgently needed. The main cause leading to poor therapeutic effect in patients with ALI is the complicated molecular mechanisms of ALI referring to activation of inflammatory responses or damage of alveolar-capillary [5]. Interestingly, some studies have pointed out the relationship of oxidative stress and AMPK/Nrf2 pathway in ALI, and these previous results provided research trend and data for ALI therapy [6, 7]. However, the detailed mechanism of AMPK/Nrf2 pathway controlling oxidative stress in ALI remain elusive, and it is urgent to conduct related study to elucidate the role of oxidative stress and AMPK/Nrf2 pathway in ALI process.

AMPK/Nrf2 pathway, an important signaling pathway controlling numerous cellular processes, which is involved in many human diseases by modulating inflammatory responses and oxidative stress [8, 9]. Given the effect of AMPK/Nrf2 signaling pathway in cellular defense against various diseases by alleviating inflammatory responses and oxidative stress, it is well-known to its seminal role in diseases process. Aberrant expression of AMPK/Nrf2 pathway can aggravate the ALI and its applications in patients with ALI. Moreover, Wu YX et al [10] has investigated the role of sophoricoside in Acute lung injury, and the results showed activating MPK/Nrf2 signaling pathway attenuates progression of ALI by reducing production of pro-inflammatory cytokines. Simultaneously, Huang XT [11] has reported the effect of Galectin-1 in MPK/Nrf2 signaling pathway, and the outcomes suggested aberrant activation of MPK/Nrf2 signaling pathway are closely associated with the occurrence and progression of ALI. However, few studies have reported the effect of AMPK/Nrf2 pathway signaling pathway regulating oxidative stress in ALI, and the mechanism of AMPK/Nrf2 signaling pathway in ALI is still elusive.

Oxidative stress [12, 13], a well-known causative factor in numerous diseases, which can lead to abnormal cellular processes by damaging the structure of proteins and nucleic acids. Considering that oxidative stress plays a key role in the occurrence and progression of ALI. Some studies [14, 15] have pointed out inhibition of oxidative stress is a potential therapeutic strategy in managing ALI process. Besides, Kong L [16] has investigated the role of oxidative stress in ALI development, the results show oxidative stress is essential to aggravate the ALI development and its corresponding complications by increasing pro-inflammatory factors. We can see that inhibiting oxidative stress exhibits great potential in ameliorating ALI and its complications. However, corresponding studies are still limit. To further investigate the mechanism of AMPK/Nrf2 pathway signaling pathway regulating oxidative stress in ALI, a vivo study is conducted.

Here, we conducted a vivo study to investigate the molecular mechanisms of AMPK/Nrf2 pathway in LPS -induced acute lung injury by mediating oxidative stress.

Methods

ALI animal model establishment

All procedures used for this animal experiment have been approved by the ethics committee of our hospital. The mice (BALB/c, male, 8 weeks) were purchased from Hunan SJA Laboratory Animal Co., Ltd. Certificate No.: SCXK2022-0006. The mouse ALI model was established via intraperitoneal injection of LPS at a dose of 10 mg/kg, and the mice in the control group were injected with the equal volume of sterile saline. Dimethyl fumarate (DMF), an activator of AMPK/Nrf2 pathway, was intraperitoneally injected into the mice in AMPK activator group before ALI model establishment according to manufacturer's instruction. Next, the mice were sacrificed under anesthetic, then the lung tissue and blood samples were obtained for the further test.

Hematoxylin-eosin (HE) staining

The lung tissue from control, model, and AMPK activator groups were fixed using formalin, followed by embedment in paraffin. Next, the obtained specimens were sectioned into 5-μm section for HE staining. The results were analyzed by a pathologist using an optical microscope.

Injury score

According to the previous studies [17], injury score was determined by the assessment of lung injury can be based on the presence and severity of alveolar cavity, septal thickening, hyaline membrane, inflammatory cell infiltration, and hemorrhage in lung tissue. These factors provide valuable information about the extent and nature of the injury, and hemorrhage. The lung tissue injury for control, model, and AMPK activator groups was evaluated by injury score.

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Measurement of lung wet/dry ratio

According to wet/dry ratio reported in a study [18], the lung tissue was excised and washed with sterile saline. Firstly, the lung wet weight involved measuring the weight of the lung tissue as soon as it was collected, without any further drying. This measurement provided the initial weight of the lung tissue. After the wet weight measurement, the lung tissue was subjected to a drying process. It was placed in an oven at a temperature of 65℃ for a duration of 96 h. then the dry lung weight was obtained. Finally, the wet/dry ratio is a calculated value that represents the amount of water present in the lung tissue relative to its dry weight. It is determined by dividing the wet weight of the tissue by its dry weight and multiplying by 100%.

Measurement of ROS level

DCFH-DA method was used to assess the production of ROS. The obtained lung tissue cells were firstly washed and then were incubated with diluted DCFH-DA for 20 min in the dark, then the cells were washed with PBS and were imaged using a confocal laser scanning microscope (Olympus FluoView FV1000, NY, USA). Finally, the cells were added into 96-well plates and then the fluorescence was determined using a spectra Max M5 (Molecular devices, CA, USA).

Measurement of IL-6 and IL-1β (pg/ml)

To assess the effect of inflammatory factors in ALI animal model, ELISA assay was used to measure the IL-6 and IL-1β level of control, model, and AMPK activator groups. IL-6 and IL-1β level was evaluated using ELISA according to described in the manufacturer' instruction.

Western blotting

The obtained protein from the lung tissue was subjected to a 10% SDS-PAGE gel. After separation, the protein bands were transferred onto a PVDF membrane. To ensure proper binding of the antibodies and accurate evaluation of protein expression, the PVDF membrane was washed with TBST for 5 min. The primary antibody used in this study was obtained from Bioworld Techmology, Inc, China and was used at a dilution of 1:2000. In addition to the primary antibody, a GAPDH antibody was also added at a dilution of 1:1000. The protein and antibody mixture were incubated overnight at 4℃. Following the incubation, the membrane was washed three times with TBST for 10 min each. This washing step helps remove any unbound antibodies and reduce background noise. A secondary antibody was then added to the membrane. The secondary antibody used in this study was obtained from Bioworld Techmology, Inc, China and was used at a dilution of 1:10,000. After addition, the membrane was blocked for 2 h at room temperature. Blocking helps prevent non-specific binding of the secondary antibody. The membrane was washed again three times with TBST for 10 min each to remove any unbound secondary antibody. To visualize the protein bands, an ECL (enhanced chemiluminescence) luminescent reagent was added. The gray value of the protein bands was analyzed to determine the level of protein expression.

Statistical analysis

Prism 8 was used to analyze the results. Measurement data and descriptive data were presented as mean ± SD and percentage, respectively. All tests repeated 3 times at least. To assess the significance of disparities between two groups, a t-test or chi-square was used, while a one-way ANOVA was employed for comparing three or more groups, and p-value < 0.05 was accepted as statistically significance.

Results

AMPK/Nrf2 participate in LPS -induced acute lung injury

To confirm improvement of LPS -induced acute lung injury by activating AMPK/Nrf2 signaling pathway, an in vivo model of ALI was established using intraperitoneal injection of LPS to administrate the mice, then detect the effect of AMPK/Nrf2 pathway in ALI animal model. The HE staining result suggested that the lung tissue in model group exhibited significantly more lung damage and increase in inflammatory cell infiltration compared to those in control group (shown in Fig. 1A). Next, the injury score of the lung tissue was measured, and the lung injury score was determined by assessing several histopathological parameters, including the presence and severity of alveolar cavity damage, septal thickening, hyaline membrane formation, inflammatory cell infiltration, and hemorrhage in lung tissue. Each parameter was scored on a scale of 0 to 4, with 0 indicating no injury and 4 indicating severe injury. The overall lung injury score was calculated by summing the scores of these parameters, providing a comprehensive measure of the extent and severity of lung injury. In Fig. 1B, the injury scores of the lung tissues from the control and model groups are presented. The results showed that the injury score in the model group was significantly higher than that in the control group, indicating more severe lung injury in the model group. Specifically, the model group displayed marked alveolar damage, notable septal thickening, extensive hyaline membrane formation, significant inflammatory cell infiltration, and pronounced

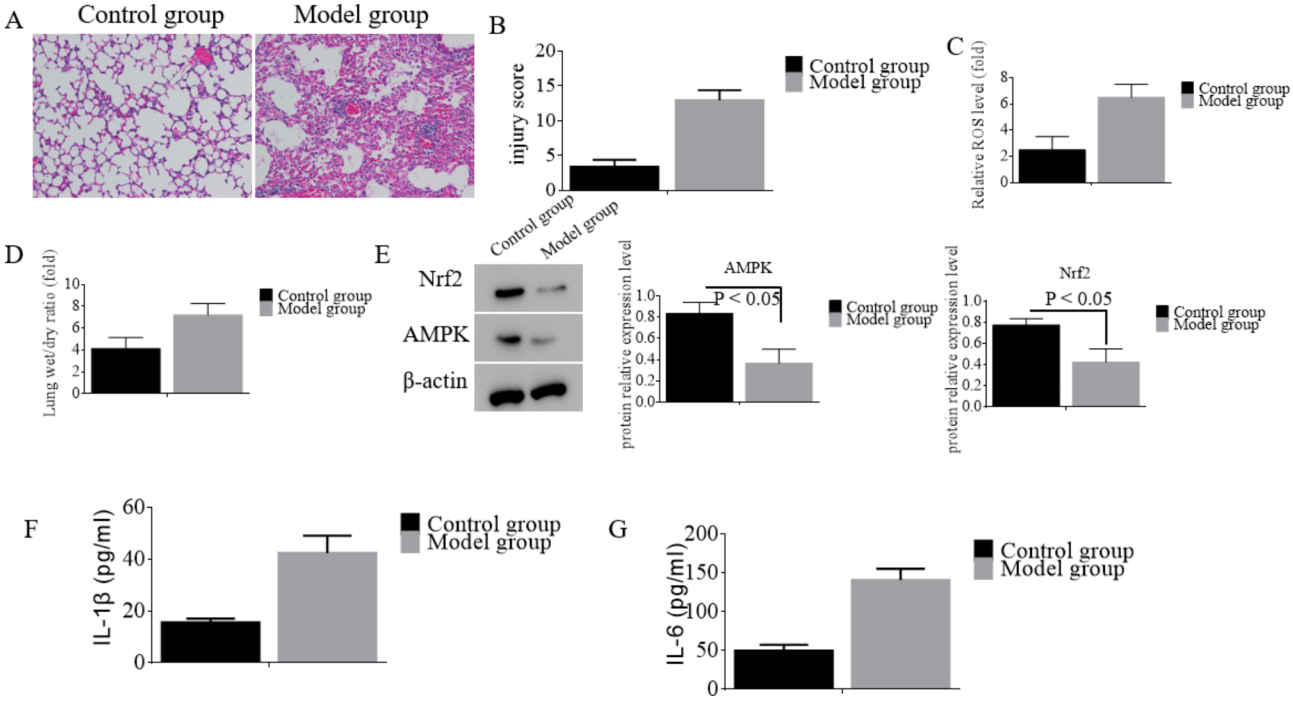

Fig. 1 AMPK/Nrf2 participate in LPS -induced acute lung injury. (**A**) HE staining was used to measure the lung tissue damage. (**B**) injury score of the lung was utilized to assess the lung tissue injury. (**C**) the production of ROS was determined using DCFH-DA. (**D**) the lung wet/dry ratio were detected. (**E**) Western blot detection of AMPK/Nrf2 pathway-related proteins including AMPK and Nrf2. (**F**-**G**) the production of IL-6 and IL-1β was determined using ELISA

hemorrhage (presented in Fig. 1B). DCFH-DA was utilized to assess the production of ROS, and these results suggested that LPS accelerated the production of ROS, and the ROS level in model was clearly increased compared to that in control group (Fig. 1C). Besides, the lung wet/dry ratio was assessed. compared to control group, the lung wet/dry ratio in model group was significantly increased (Fig. 1D). Western blot was used to detect AMPK/Nrf2 pathway-related proteins including AMPK and Nrf2 proteins, these results show that LPS obviously reduced the protein level of AMPK and Nrf2 (Fig. 1E). Subsequently, the inflammatory factors including IL-6 and IL-1β were calculated using ELISA. Compared to control group, IL-6 and IL-1β level in model group were significantly increased. These findings demonstrated that AMPK/Nrf2 pathway participates in LPS -induced acute lung injury.

Activating the AMPK/Nrf2 pathway alleviated LPS -induced acute lung injury

To investigate the effect of activating AMPK/Nrf2 signaling pathway in LPS -induced acute lung injury, the mouse was intraperitoneally injected into the mice with AMPK activator DMF before ALI model establishment, then an in vivo model of ALI was established. Subsequently, assess the role of AMPK/Nrf2 pathway in ALI animal model. The HE staining result suggested that the lung tissue in model group exhibited significantly more lung damage and increase in inflammatory cell infiltration compared to those in AMPK activator and control group (shown in Fig. 2A). In Fig. 2B, the injury scores following treatment with Dimethyl fumarate (DMF) were evaluated. The results demonstrated a significant reduction in the injury scores in the treatment group compared to the model group, suggesting that DMF alleviated the lung injury by reducing oxidative stress and inflammation. (presented in Fig. 2B). DCFH-DA was utilized to assess the production of ROS, and these results suggested that LPS accelerated the production of ROS, while AMPK activator DMF inhibited the production of ROS. the ROS level in model was clearly increased compared to that in control and AMPK activator group (Fig. 2C). Besides, the lung wet/dry ratio was assessed. compared to AMPK activator and control group, the lung wet/dry ratio in model group was significantly increased (Fig. 2D). Western blot was used to detect AMPK/Nrf2 pathway-related proteins including AMPK and Nrf2 proteins, these results show that LPS obviously reduced the protein level of AMPK and Nrf2, while DMF reversed the effect of LPS in the protein level of AMPK and Nrf2 (Fig. 2E). Subsequently, the inflammatory factors including IL-6 and IL-1β were calculated using ELISA. Compared to AMPK activator and control group, IL-6 and IL-1β level in model group were significantly increased (Fig. 2F-G). These findings demonstrated that activating AMPK/Nrf2 pathway alleviated LPS -induced acute lung injury.

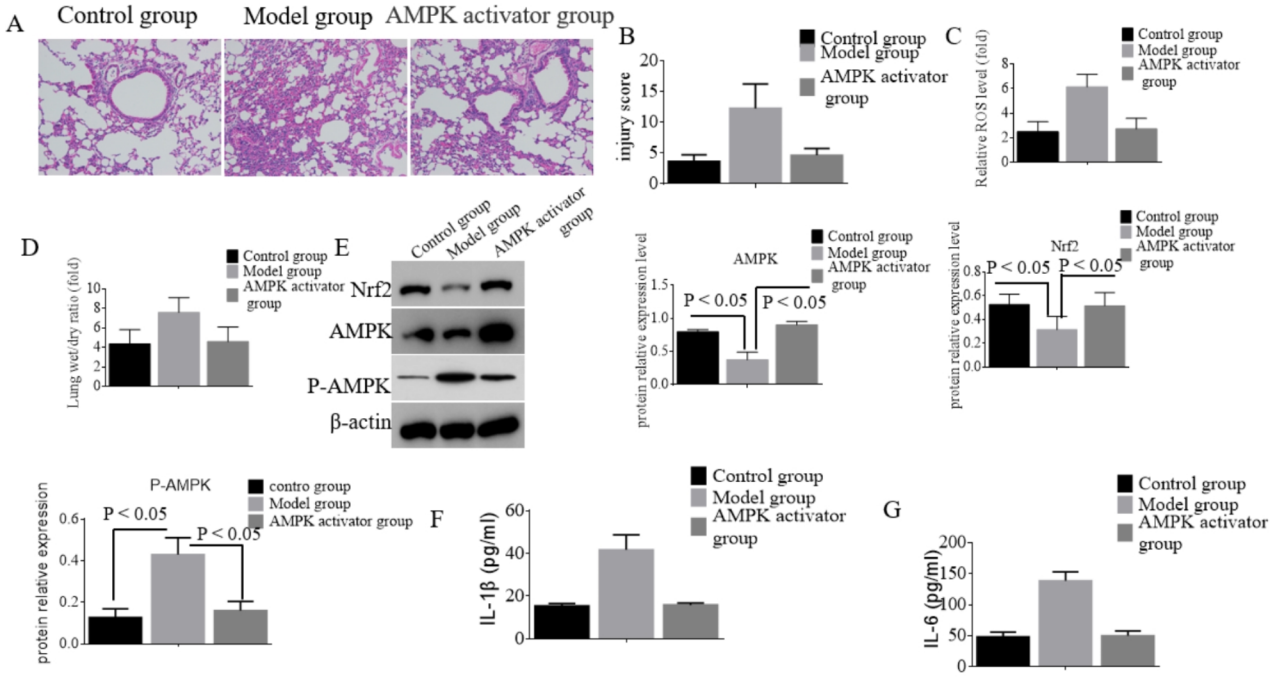

Fig. 2 Activating the AMPK/Nrf2 pathway alleviated LPS -induced acute lung injury. (**A**) HE staining was used to measure the lung tissue damage. (**B**) injury score of the lung was utilized to assess the lung tissue injury. (**C**) the production of ROS was determined using DCFH-DA. (**D**) the lung wet/dry ratio were detected. (**E**) Western blot detection of AMPK/Nrf2 pathway-related proteins including AMPK and Nrf2. (**F**-**G**) the production of IL-6 and IL-1β was determined using ELISA

Discussion

Numerous diseases-related ALI contributed to high mortality [19]. Currently, various therapeutic methods have been used to prevent ALI process, but ALI cannot be cured and only corresponding symptoms were relieved, and complicated mechanism of ALI led to poor therapeutic effect. More and more studies [10, 20] have indicated that oxidative stress and AMPK/Nrf2 pathway were associated with the occurrence of ALI, their role and mechanism in ALI, yet, were still unclear. Further study should be carried out to elucidate the mechanism of ALI. The results in this study mainly investigate the molecular mechanisms of AMPK/Nrf2 pathway in LPS -induced acute lung injury by mediating oxidative stress. These outcomes suggested that the activation of AMPK/Nrf2 pathway can prevent the ALI process via reducing the oxidative stress.

AMPK/Nrf2 pathway, an important signaling pathway controlling numerous cellular processes, which is involved in various diseases by modulating inflammatory responses and oxidative stress. Currently, some trials have reported the effect of AMPK/Nrf2 signaling pathway in ALI process. A trial by Wu YX [21] has confirmed the role and mechanism of Ethyl ferulate in ALI process, concluding that activating of AMPK/Nrf2 pathway alleviating LPS-induced AMI. Additionally, an in vivo study by Gan Q [7] also pointed out the effect of AMPK/Nrf2 pathway in LPS-induced AML mice, the results suggested the protect effect of activating AMPK/Nrf2 pathway in LPS-induced AML mice. Our results suggested that activation of AMPK/Nrf2 pathway can alleviate the occurrence of ALI, these results were consistent with those described in related studies.

Currently, the detailed mechanism of oxidative stress in ALI remained elusive. Present trial has elucidated the effect of oxidative stress in ALI and the results suggested that down-regulation of ROS had a protective effect in LPS-induced mice. Guo Y [22] has investigated the role of oxidative stress in ALI, their results showed that antioxidative stress can protect against ALI via down-regulation of inflammation. However, the mechanism of AMPK/Nrf2 pathway regulating oxidative stress in ALI was still unclear. Our results suggested activation of AMPK/Nrf2 pathway can ameliorate LPS-induced AMI mice via inhibiting the oxidative stress and corresponding inflammatory factor level. The results indicated AMPK/Nrf2 pathway regulating oxidative stress was correlated with ALI progress.

A limitation of present study was that we only conducted an in vivo study to investigate that the molecular mechanisms of AMPK/Nrf2 pathway regulating oxidative stress in LPS-induced AMI mice, and further clinical study was needed to manage.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13019-024-03020-2.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The ethic approval was reviewed and approved from The Obstetrics and Gynecology Hospital Affiliated to Fudan University.

Consent for publish
All of the authors have consented to publish this research.

Competing interests
The authors declare no competing interests.

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