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Original Article

Inhibition of RXRA-mediated PLA2G2A improves delirium in COPD mice by regulating endoplasmic reticulum stress pathway and inhibiting cell apoptosis

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Abstract

Delirium is a common psychiatric complication of chronic obstructive pulmonary disease (COPD). The relief of delirium is considered one of the beneficial ways to treat COPD. However, there are currently no specific drugs that alleviate delirium in COPD patients. Our research aimed to elucidate the specific mechanisms underlying delirium in COPD mice, while also seeking more effective therapeutic targets. In our study, bioinformatics analysis and qRT PCR were used to identify key factors in the development of delirium in COPD animal models. Open field and elevated plus maze tests were used to detect delirium in mice. Tunel staining and HE staining were used to analyze the apoptosis of mouse hippocampus cells. EdU and CCK-8 experiments were used to analyze PC-12 cells vitality and proliferation. JASPAR online database, dual luciferase reporting experiments, ChIP experiments, and IF staining were used to analyze the interaction between RXRA and PLA2G2A. RXRA is highly expressed in the brain tissue of COPD mice with delirium symptoms. The downregulation of RXRA inhibits the delirium state in COPD mice. This is mainly due to the reduction of endoplasmic reticulum stress and cell apoptosis by inhibiting the expression of RXRA. In addition, we also confirmed that RXRA is a transcription factor of PLA2G2A. RXRA has an inhibitory effect on the expression of PLA2G2A. *In vitro* experiments have confirmed that inhibition of the RXRA/PLA2G2A axis reduces cell apoptosis, thereby alleviating the occurrence and development of delirium in COPD mice. Inhibition of the RXRA/PLA2G2A axis reduces endoplasmic reticulum stress and cell apoptosis. This process alleviates the development of delirium in COPD mice.

Keywords: COPD, Delirium, PLA2G2A, RXRA

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a common chronic bronchitis. According to the latest data, approximately 2.7 million people died of COPD in 2022, ranking fourth in the world, and this number is gradually increasing [1]. Histological research has confirmed that COPD development is mainly due to the long-term effects of inflammatory reactions induced by harmful particles on the small airways. Therefore, long-term smoking is the most common factor inducing COPD, accounting for approximately 50% of the population. In addition, genetics, air pollution, and recurrent asthma are also contributing factors to COPD [2]. Usually, the early clinical manifestations of these patients are pulmonary symptoms (such as coughing, shortness of breath, and difficulty breathing). When the condition further develops, the patient develops hypoxemia and Hypercapnia. Among them, severe patients may also experience mental and neurological disorders, with specific clinical manifestations including headache, dizziness, restlessness, and hallucinations. Some patients even exhibit a state of delirium [3]. The existing treatment methods for late-stage COPD patients still focus on improving lung function. However, this treatment plan lacks a personalized diagnosis and treatment plan for the development of COPD and its complications.

Delirium is a brain syndrome caused by central system dysfunction and a common clinical complication [4]. It induces disorders in patients' consciousness, thinking, and perception. Clinical studies have confirmed that the incidence of delirium in middle-aged and elderly COPD patients is high, and the mortality rate of COPD patients with delirium is higher than that of ordinary patients [5]. Alleviating COPD in patients also effectively alleviates their delirium state. However, the current treatment methods for COPD patients with concurrent delirium mainly focus on alleviating their mental state through medication or non-medication methods. However, few studies have focused on analyzing the molecular mechanisms underlying its occurrence and development.

RXRA is one of the nuclear receptors like Retinol X receptors (RXR), which is mainly located on chromosome 9q34.2. Its abnormal expression is often associated

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with diseases such as acute myeloid leukemia, malnutrition, and Alzheimer's disease [6-8]. This is mainly due to the involvement of RXRA as a retinoic acid X receptor in the vitamin D pathway. This pathway is also related to endoplasmic reticulum stress (ERS) and lipid metabolism in the body [9, 10]. Phospholipase A2, group IIA (PLA2G2A) is also a protein that participates in lipid metabolism. PLA2G2A is a secretory Phospholipase with a molecular weight of 16kDa. PLA2G2A is often considered to be associated with the occurrence of diabetes, lymph node and pancreatic cancer [11-13]. However, few studies have discussed whether the abnormal expression of RXRA and PLA2G2A is associated with delirium in COPD patients.

Our study's purpose was to find regulatory effects of RXRA on delirium status in COPD mice. Our work found significant overexpression of RXRA in the brain of delirious COPD mice. We speculate that RXRA mediates ERS and inhibits apoptosis through PLA2G2A signaling pathway. This process effectively alleviates the delirium state of COPD mice.

2. Materials and methods

2.1. Cell culture

A549, 16HBE, HFL1, HBF, and PC-12 cell lines were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. Cells were cultured under same standard conditions (5% CO_2 and 37°C). Cells were cultured in DMEM complete culture medium (DMEM base medium: total body serum: penicillin/streptomycin=89:10:1). When the cell grows to around 85% convergence, 0.25% (w/v) trypsin solution is used for cell passage.

2.2. Cell transfection

Short hairpin RXRA (shRXRA), short hairpin PLA2G2A (PLA2G2A), overexpression RXRA (shRXRA), overexpression PLA2G2A (PLA2G2A) and its negative control (sh-NC and vector) were purchased from GenePharma (Shanghai, China). Transfections were performed using Lipofectamine 2000 reagent (Invitrogen, California, USA), according to the manufacturer's instructions.

2.3. qRT-PCR

A549, 16HBE, HFL1, HBF cell lines and PC-12 cells were extracted total RNA by TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The addition of gDNA Eraser (TaKaRa, Liaoning, China) facilitates the removal of genomic DNA. SuperScript IV (ThermoFisher) was used to reverse transcribe total RNA. One Step SYBR® PrimeScript RT-PCR Kit (TaKaRa) was used to perform qRT-PCR analysis on the ProFlex™ PCR system (ThermoFisher). PCR primers are listed in Table 1. GAPDH is the internal control.

2.4. Western blot

RIPA Buffer Concentrate (Cayman Chemical, State of Michigan, USA) was used to lysed different cell lines. The total protein was transferred to SDS-PAGE for constant pressure electrophoresis for 120 min. Isolated proteins were transferred to the Immobilon-E-PVDF membrane (Merck, Darmstadt, Germany). The membrane was incubated with primary antibodies at 4°C for 12 h. The secondary antibodies were incubated for 2h. Enhanced chemiluminescence was used to observe the proteins. The antibodies used in our work are shown below: anti-PERK (ab229912, Abcam, Cambridge, UK), anti-IRE1α (ab235171, Abcam), anti-ATF6 (ab227830, Abcam), anti-CHOP (15204-1-AP, Proteintech Group, Inc., China, Wuhan), anti-Caspase12 (ab62484, Abcam), anti-RXRA (ab6125001, Abcam), anti-PLA2G2A (ab23705, Abcam), Alexa Fluor® 488 (ab150079, Abcam), Alexa Fluor® 647 (ab150077, Abcam) and GADPH (ab9485, Abcam).

2.5. Immunofluorescence (IF) staining

Cells were washed with PBS, fixed with paraformaldehyde, permeabilized with 0.5% triton X-100 (Beyotime), blocked (bovine serum albumin) and then incubated with anti-RXRA and anti-PLA2G2A overnight at 4°C. After incubation with Alexa Fluor® 488 (green) and Alexa Fluor® 647 (red), DAPI (Beyotime) (blue) was used to stain nuclei. Finally, cells images were obtained using confocal microscopy (Leica, Germany).

Table 1. The primers used in our work.

Name	Sequence (5'-3')
RXRA-F	ATGGACACCAAACATTTCCTGC
RXRA-R	CCAGTGGAGAGCCGATTCC
PLA2G2A-F	CTATGCCTTCTATGGATGCCAC
PLA2G2A-R	CAGCCGTTTCTGACAGGAGT
PERK-F	TCCCCTAGATCCCCTGAACTT
PERK-R	TTTCGAGCTGAGTGCTCTACA
IRE1α-F	AGTGGATCTAAATGAGGGCAGT
IRE1α-R	GTTTCCAGGCGCAGCTTAC
ATF6-F	TCTCCTCGGATGAGCAGGG
ATF6-R	CTTCCCGAAGGGGTTCCAT
CHOP-F	TTGCCCTCTTATTGGTCCAGC
CHOP-R	TAGCGACTGTTCTGTTCCCAC
Caspase12-F	TAGGGGAAAGTGCGAGTTTCA
Caspase12-R	GGGCCAATCCAGCATTTACCT
GAPDH-F	ATTGTTGCCATCAATGACCC
GAPDH-R	AGTAGAGGCAGGGATGATGT

2.6. Animal

Male C57BL/6 mice were purchased from the National Rodent Seed Center (Shanghai, China). Mice weights are ≥200 and <250 g. Mice were fed in a feeding box at 25℃ with humidity was 50-60%. The light and dark cycle time of the feeding box was 12:12 hours. Mice freely obtained feed and water. All procedures involved were strictly approved by animal protection and utilization committee of Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University and performed following *the Animals (Scientific Procedures) Act 1986.*

Mice were infected twice a week by intratracheal instillation 5 μ G AAV-RXRA, si-RXRA, si-PLA2G2A, lv-NC or sh-NC (GenePharma). Two weeks after successful infection, we prepared a COPD model as described earlier [13]. Mice were confined in a closed box. We created the box environment where rats were repeatedly exposed to *Klebsiella pneumoniae* and tobacco. The frequency of infection in mice is 2 times a day, lasting for 2 hours each time. The control group of mice was untreated (12 mice). Four months later, we established a delirium state in mice through chemical induction [2]. In short, we mainly injected 200 μ g/kg Lipopolysaccharide into the abdominal cavity of mice. After 4 days, we conducted behavioral experiments on mice. Then, the mice were euthanized. We collected mouse plasma and Hippocampus samples for subsequent experiments.

2.7. Open-field test

The XR-XL303 joint open field experimental system (90 cm × 90 cm × 40 cm; Xinruan Information Technology, Shanghai, China) was used for open field testing. It was divided into 9 areas of the same area (30 cm× 30 cm). The experimental mouse was put in the central area and allowed to freely explore 9 equal areas for 10 minutes. SuperMaze software 3.0 (Xinruan Information Technology) was used to record the movement and time of mice in each region.

2.8. Elevated plus maze test

KW-GJ maze system (KEWBASIS, China, Nanjing) was used for the elevated plus maze test. We observed and recorded the open arms of mice within 5 minutes. We use the time spent by mice on closing and opening their arms as a scoring criterion.

2.9. Hematoxylin-eosin staining

The mice tissues (heart and lung) were fixed using Hematoxylin-Eosin staining (Klamar, China, Shanghai), paraffin-embedded and sliced into 5μm. Hematoxylin and eosin were used to stain sections. A BX51 optical microscope was used to observe.

2.10. Pulmonary function testing

The pulmonary function of mice was mainly assessed by: 1. Peak expiratory flow (PEF) in the third second. 2. Forced expiration volume (FEV0.3)/forced expiration capacity (FVC). The PFT animal lung function testing system (TOW-INT TECH, China, Shanghai) was used to detect FEV0.3, FVC, and PEF in mice.

2.11. TUNEL inspection

Apoptosis of neurons in the hippocampus was assessed in sections by TUNEL staining. The cells were incubated with TUNEL mixture. Sections were washed three times and stained with DAPI. Sections were observed under fluorescence microscope. Flow cytometry is used to quantify the results.

2.12. CCK-8

According to the manufacturer's protocol, the transfected PC-12 cell lines were incubated with 10 μL CCK-8 solution (Beyotime) in 100 μL DMEM medium containing FBS. Absorbances were measured at 450 nm.

2.13. EdU

96-well plates were used to culture PC-12 cells. Their densities were 1×10^4 cells/ well. Wells were stained with 100 μL EdU solution (red) or DAPI (blue). After washing with PBS, 4% paraformaldehyde was used to fix cells for 30 min. Each well was added with 100 μL 0.5% TritonX-100 penetrant. Then, the wells were incubated in a decolorizing shaker for 10 min. After cleaning, the staining was photographed under fluorescence microscopes.

2.14. Dual-luciferase report experiment

RXRA (WT) and Mutant RXRA (MUT) fragments were amplified and inserted into the pGL3-hRluc plasmid. They were co-transfected with siPLA2G2A to PC-12 cell lines. DLR™ Assay System Kits (Lumiprobe, Shanghai, China) were used to analyze luciferase activity.

2.15. Chromatin immunoprecipitation (ChIP)

We used ChIP Kit (Axl-Biotech, China). In short, after cell cross-linking and inactivation DNA was cut into fragments by ultrasound treatment. The supernatant and normal rabbit IgG antibodies are negative controls. Immunoprecipitated protein chromatin complexes were co-incubated with protein A/G agarose beads for 2 hours before reverse crosslinking at 65 ℃. After DNA purification, PLA2G2A expression was measured by qRT-PCR.

2.16. Bioinformatics analysis

GSE475 and GSE3320 datasets were obtained from the GEO database to assess the expression patterns of ASD-related differentially expressed genes using R language. The Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/) drew the Venn diagram of down-regulated genes from the GSE475 and GSE3320 datasets. JASPAR (http://jaspar.genereg.net/) online analysis websites predicted target genes of transcription factor RXRA.

2.17. Statistical analyses

The SPSS16.0 software (Chicago, USA) was used to conduct data analysis. Data were presented as mean ± standard deviation from at least three independent replicates. Statistical differences between groups were calculated using one-way ANOVA. Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Low-level expression of RXRA in COPD

To obtain expression patterns of COPD-associated differentially expressed genes, we analyzed the GSE475 and GSE3320 datasets. As shown in Figures 1A, B, 125 down-regulated genes in GSE475 dataset and 198 in GSE3320 were screened. Venn diagram showed that there were 7

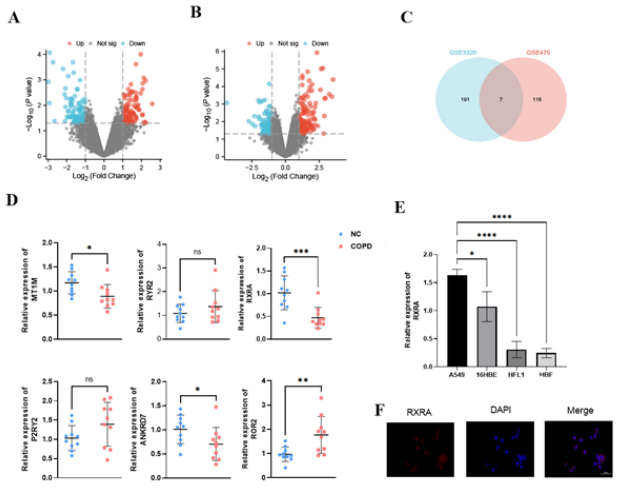

Fig. 1. Low expression of RXRA in COPD animal models and cells. Volcanic maps show differentially expressed genes in the GSE475 dataset(A) and GSE3320 dataset(B). (C) Venn diagram showed that differentially expressed genes were found in both datasets. (D) MT1M, RYR2, RXRA, P2RY2, ANKRD7 and ROR2 expression in COPD mice blood samples. (E)RXRA expression in cell lines. (F) IF staining was used for nuclear localization of RXRA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

genes in the intersection set of these down-regulated genes (Figure 1C). Next, qRT-PCR detected gene expression in blood from mice with COPD, and the results showed that RXRA, MT1M and ANKRD7 were all low expressed in mice with COPD (Figures 1D-G). Among them, RXRA was the most significantly low expressed in COPD mice (Figure 1D). RXRA also had low expression in HFL1 and HBF cell lines (Figure 1E). As shown in Figure 1F, RXRA was located in the nucleus. Based on these findings, we speculated that RXRA is a key factor affecting COPD.

3.2. Inhibition of RXRA expression alleviates delirium in COPD rats

To analyze the effect of RXRA on delirium status in COPD mice, we constructed a mouse model of COPD and delirium. Firstly, we analyzed the expression of RXRA in different groups of rat brain tissues. As shown in Figure 2A, the expression of RXRA in the brain tissue of COPD rats decreased. Interestingly, we found that compared to other groups, the COPD+Delirium group had a higher expression level of RXRA in the brains of rats. Secondly, we analyzed the symptoms of COPD in different groups of mice. The HE staining results showed that compared to the Con group, other groups exhibited phenomena such as detachment and lodging of cilia from airway epithelial cells, obstruction of the airway by inflammatory secretions in the terminal small airway lumen, thickening of airway wall, and formation of pulmonary bullae. And the mean linear intercept of the COPD+Delirium group is the highest (Figure 2B). The lung function test of mice also showed that the COPD+Delirium group had the lowest FEV of 0.3/FVC and PEF. Inhibiting RXRA expression alleviated the decrease in lung function (Figure 2C). Subsequently, we tested the cognitive and social behavior of different groups of mice. The results of the Open field test showed that the time span in chamber and frequency of crossing into the center of the COPD+Delirium group mice were significantly lower than those of the Con and COPD groups. However, inhibiting of RXRA expression increased these indicators (Figure 2D). The results of the Elevated Plus maze test showed that the time spent in open arms of COPD+Delirium group mice was significantly higher than that of Con and COPD groups. However, inhibiting of RXRA expression reduced this time (Figure 2E). Finally, we analyzed the causes of delirium in COPD mice. Western blot analysis of mouse brain tissue showed that compared to the Con and COPD groups, the COPD+Delirium group had the highest expression of PERK, IRE1α, CHOP, and Caspase-12, while the expression of ATF6 was the lowest. However, inhibiting RXRA expression altered this expression level (Figure 2F). We conducted validation through in vitro experiments. We suppressed the expression of RXRA in PC-12 cells (Figure 2G). The TUNEL detection results showed that when the expression of RXRA was inhibited, the apoptosis rate of hippocampal cells was significantly reduced (Figure 2H). The results of flow cytometry showed that when the expression of RXRA was low, the apoptosis rate of PC-12 cells was significantly reduced (Figure 2I). In short, inhibiting the expression of RXRA inhibits brain cell apoptosis through the PERK/IRE1α/CHOP pathway, thereby alleviating delirium in COPD mice.

3.3. The binding effect of RXRA and PLA2G2A

To further elucidate the mechanism by which RXRA alleviates delirium in COPD mice, we analyzed the binding protein of the transcription factor RXRA through

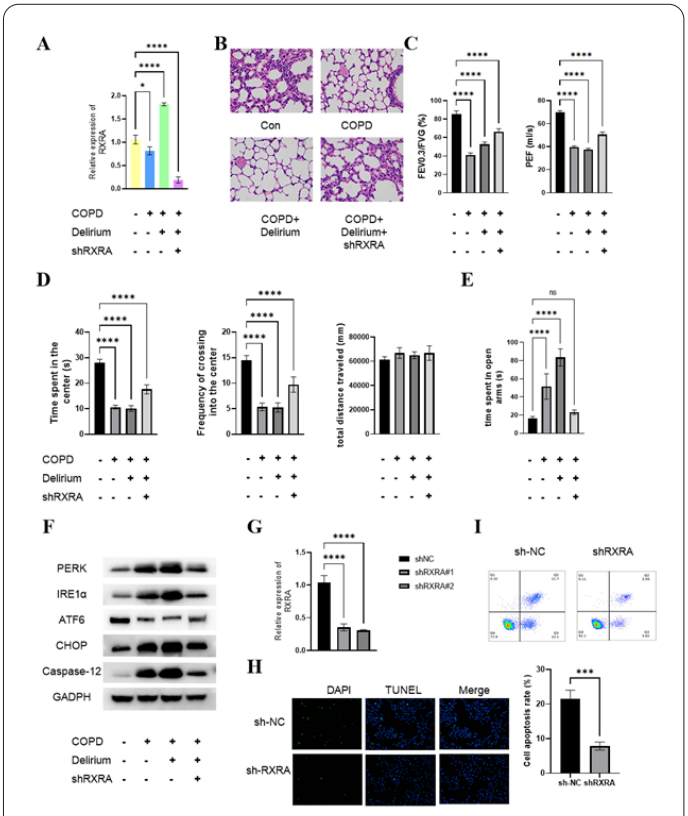

Fig. 2. Inhibition of RXRA expression alleviates delirium in COPD mice. (A) RXRA expression in each group of models. (B and C) HE staining and lung function analysis were used to evaluate COPD symptoms. (D and E) Open field and elevated plus maze tests were used to evaluate the delirium state of mice. (F)Western blot was used to detect PERK, IRE1α, ATF6, CHOP, and Caspase-12 expression in mouse brain tissue. (G) Detection of transfection efficiency of RXRA. (I)TUNEL detection was used to evaluate cell apoptosis. (H) Flow cytometry was used to evaluate cell apoptosis *in vitro*. **p<0.01, ***p<0.001, ****p<0.0001.

JASPAR. We found binding bits between the transcription factor RXRA and PLA2G2A (Figure 3A). The experimental results of dual-luciferase report experiment and CHIP confirmed the interaction between RXRA and PLA2G2A (Figure 3B, C). IF staining also showed that both RXRA and PLA2G2A were enriched in the nucleus (Figure 3D). We found a decrease in PLA2G2A expression in PC-12 cells with low expression of RXRA (Figure 3E). The expression of PLA2G2A in cells and brain tissue is also correlated with RXRA (Figure 3F, G).

3.4. Low expression of RXRA inhibits PLA2G2A-mediated cell apoptosis

To confirm whether RXRA mediates the regulation of cell apoptosis by PLA2G2A, we successfully suppressed the expression of PLA2G2A in PC-12 (Figure 4A). CCK-8 and EdU assays showed that inhibiting the expression of PLA2G2A promoted the increased cell proliferation rate due to low RXRA expression (Figure 4B, C). TUNEL detection confirmed that low expression of PLA2G2A inhibited the reduced cell apoptosis rate due to low expression of RXRA (Figure 4D). The results of Western blot analysis showed that inhibiting the expression of PLA2G2A also inhibited the expression of PERK, IRE1α, CHOP, and Caspase-12 (Figure 4E). In conclusion, RXRA inhibits ERS and apoptosis of PC-12 cells by mediating PLA2G2A.

3.5. RXRA mediates PLA2G2A to alleviate delirium in COPD mice

To confirm the involvement of the PLA2G2A/RXRA pathway in the regulation of delirium in COPD patients, we reconstructed a COPD mouse model. As shown in Figure 5A, we successfully suppressed the expression of RXRA and PLA2G2A in the COPD rat model. Compared to the COPD group, the mean lining interval of rats in the COPD+shRXRA and COPD+shRXRA+shPLA2G2A groups increased. The results of the Open field test showed that the COPD+shRXRA+shPLA2G2A group of rats had higher time spent in the chamber and frequency of crossing into the center compared to the COPD and COPD+shRXRA groups (Figure 5D). The results of the Elevated plus maze test showed that the time spent in open arms of the COPD+shRXRA+shPLA2G2A group rats was lower than that of the COPD and COPD+shRXRA groups (Figure 5E).

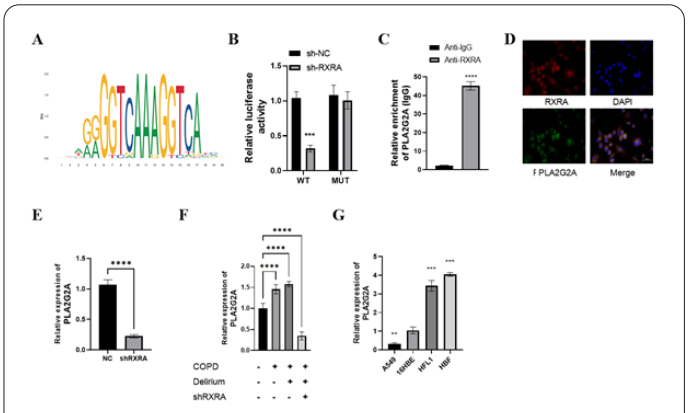

Fig. 3. Binding effect of RXRA and PLA2G2A. (A) The online software JASPAR predicts the target protein of RXRA. The interaction between RXRA and PLA2G2A was verified by (B and C) dual-luciferase report experience and CHIP. (D) IF staining was used for the nuclear localization of RXRA and PLA2G2A. PLA2G2A expression in mice(F) and cells (E and G). ***p<0.001, ****p<0.0001.

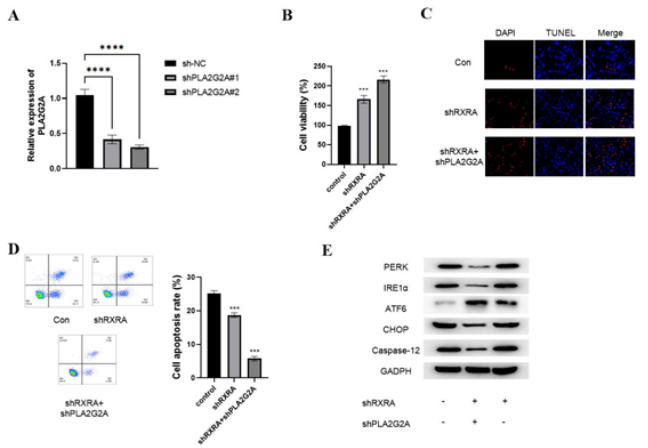

Fig. 4. RXRA inhibits ERS and apoptosis by mediating PLA2G2A. (A) Detection of transfection efficiency of PLA2G2A. (B) CCK-8 analysis was used to detect cell viability. (C) EdU analysis is used to detect cell proliferation. (D) Flow cytometry was used to analyze cell apoptosis. (E)Western blot was used to detect PERK, IRE1α, ATF6, CHOP, and Caspase-12 expression in mouse brain tissues. **p<0.01, ***p<0.001, ****p<0.0001.

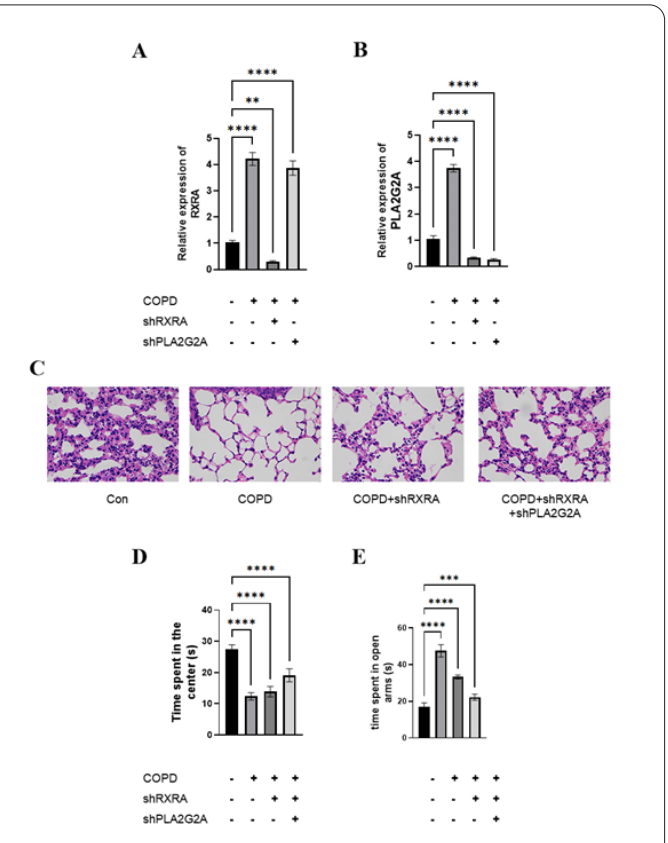

Fig. 5. RXRA mediates PLA2G2A to alleviate delirium in COPD mice. (A and B) RXRA and PLA2G2A expression in various mouse models. (C) HE staining was used to evaluate the symptoms of COPD in mice. Open field (D) and elevated plus maze (E) tests were used to evaluate the delirium state of mice. ***p<0.001, ****p<0.0001.

4. Discussion

There are multiple causes of delirium in COPD patients, and there is currently no specific drug treatment available [14]. Clinical studies have confirmed that alle-

viating the onset of delirium in patients is often beneficial for the treatment of COPD [5]. Our study confirmed that overexpression of RXRA mediates PLA2G2A to improve delirium in COPD mice. This effect is mainly through regulating the ERS pathway to inhibit apoptosis.

ERS is an adaptive regulation behavior that is conducive to the survival of the body. It is widely involved in the progress of diseases, such as fatty liver, cardiovascular diseases and breast cancer [15-17]. The research of Das *et al*. [18] shows that ERS affects cell apoptosis by activating unfolded protein response. Zhang *et al*. [19] found that ERS promotes cell apoptosis in COPD mice. Our research has found a new molecular mechanism that ERS affects apoptosis in mice with COPD and then affects delirium. In addition, vitamin D pathway participates in regulating the body's growth and development, inflammatory response, and immune processes [20]. The research of Ahmad et al. confirmed that vitamin D pathway down-regulates ERS to alleviate cell apoptosis and alleviate acute lung injury [10]. Marques *et al*. [21] also confirmed that vitamin D pathway regulates ERS to alleviate breast cancer. RXRA is considered a key transcription factor in the vitamin D pathway and is believed to function by binding to vitamin D receptors to form heterodimers [22]. The clinical study of Jolliffe *et al*. [23] preliminarily analyzed the Single-nucleotide polymorphism of RXRA in COPD. Our study further confirms that the abnormal expression of RXRA is associated with COPD development and complications. Li *et al*. 's study confirmed that RXRA is highly expressed during the treatment of brain injury [24]. Our research results also confirm that RXRA is highly expressed in the acute encephalopathy syndrome of COPD. In addition, our study also confirmed that inhibiting the expression of RXRA greatly alleviated the delirium state in COPD rat models.

PLA2G2A belongs to phospholipase A2 family [11]. The clinical investigation of Zhu *et al*. [25] confirmed that enhancement of phospholipase A2 family promotes hip Joint replacement patients delirium development. PLA-2G2A has abnormal expression in various complications of diseases, such as hepatitis B virus, multi-system inflammatory syndrome in children, and total growth restriction [6, 26]. In COPD, abnormal expression of PLA2G2A has also been confirmed. Mohammadtursun *et al*. [27] found that PLA2G2A is highly expressed in COPD mice. This is consistent with the results of animal models and cell line testing. Liu *et al*. [28] found that PLA2G2A participates in the progression of cerebral ischemic stroke. Our study found for the first time that PLA2G2A participates in the improvement of delirium state. In addition, we also discovered RXRA as a transcription factor for PLA2G2A. The inhibition of RXRA reduces the expression of PLA2G2A, which improves the delirium state of the COPD rat model.

5. Conclusion

In summary, the results of this experiment confirm that RXRA, as a transcription factor of PLA2G2A, regulates the ERS pathway and inhibits cell apoptosis. The effect of this process on improving delirium status in COPD mice. Although our work has achieved meaningful results, there are still some limitations, the most significant being the lack of clinically validated efficacy or safety. Our research contributes to a deeper understanding of the delirium status of COPD patients and provides rationale for the development of treatment strategies and drugs.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

We have received approval from the Ethics Committee of Affiliated Mental Health Center & Hangzhou Seventh People's Hospital, Zhejiang University School of Medicine and Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

ZT conducted the experiments and wrote the paper; WG conceived, designed the study and revised the manuscript.

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