



Resveratrol attenuates inflammation and apoptosis through alleviating endoplasmic reticulum stress via Akt/mTOR pathway in fungus-induced allergic airways inflammation

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ABSTRACT

Background: Resveratrol has shown pleiotropic effects against inflammation and oxidative response. The present study aimed to investigate the effects and mechanisms of resveratrol on fungus-induced allergic airway inflammation.

Methods: Female BALB/c mice were injected intraperitoneally with *Aspergillus fumigatus* (Af) extract emulsified with aluminum on day 0 and 7 and intranasally challenged with Af extracts on day 14 and 15. Resveratrol or dexamethasone or a vehicle was injected intraperitoneally 1 h before each challenge. Mice were sacrificed for serum, bronchoalveolar lavage fluid (BALF), and lungs 24 h after the last challenge. The control group was administered with saline. BEAS-2B was used for the experiments in vitro that Af-exposed airway epithelial cells. **Results:** Resveratrol and dexamethasone attenuated the airway inflammation and eosinophilia, and reduced not only the production of IL-4, IL-5, and IL-13 in the BALF and lung tissues but also the mRNA levels of lung IL-6, TNF- α , and TGF- β induced by Af challenge ($P < 0.05$). Furthermore, Af-induced lung endoplasmic reticulum (ER) stress-related proteins PERK, CHOP, and GRP78 and the apoptosis markers including cleaved caspase-3 and cleaved caspase-7 were both suppressed significantly by resveratrol ($P < 0.05$). In vitro, activation of ER stress and the Akt/mTOR pathway in Af-exposed BEAS-2B cells were effectively ameliorated by resveratrol. Inhibition of the Akt/mTOR pathway using LY294002 suppressed the ER stress while ER stress inhibitor 4-PBA decreased the apoptosis in Af-exposed BEAS-2B cells.

Conclusions: Our findings collectively revealed that resveratrol alleviated the Af-exposed allergic inflammation and apoptosis through inhibiting ER stress via Akt/mTOR pathway, exerting therapeutic effects on the fungus-induced allergic lung disorder.

1. Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a complex pulmonary disorder caused by allergic responses to the ubiquitous fungal organism *Aspergillus fumigatus* (Af) [1]. ABPA is characterized by eosinophilia, Th2-type polarized responses, elevated serum IgE, and high bronchial hyperresponsiveness, affecting approximately 4.8 million people worldwide [2,3]. The principles in the mainstay therapy of ABPA include glucocorticoids and anti-fungal agents that help

suppress immune reactions and attenuate fungal burden [1,4]. However, not all patients respond successfully to standard treatment and about half of patients experience relapse [5,6]. Nowadays, a poor understanding of the pathogenesis of ABPA hinders the development of new therapy.

Resveratrol (3, 4', 5-trihydroxystilbene) is a polyphenolic compound widely distributed in grapes, cranberries, peanuts, and red wine [7]. It has multiple biological effects, such as anti-oxidation, anti-inflammation, cardiovascular and neuroprotective protection [8]. Resveratrol has

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also been found to be a potential therapeutic agent for lung diseases [9], for example, resveratrol could alleviate airway inflammation, remodeling, eosinophilia, and mucus hypersecretion in asthmatic mouse models challenged with OVA or HDM, and protected airway epithelial against injury and apoptosis induced by HDM through inhibiting DNA damage [10–15]. In addition, PI3K/Akt/mTOR signaling was suggested as an important target for resveratrol and allergic inflammation [16,17]. However, whether resveratrol affects Af-induced allergic lung inflammation remains unknown.

Endoplasmic reticulum (ER) serves as a protein-folding factory

essential for biosynthesis, trafficking, and posttranslational modification of functional proteins [18]. Accumulation of misfolded or unfolded proteins in ER will trigger ER stress, at which point cells initiate the unfolded protein response (UPR) to overcome cellular damage [18–20]. There are three transmembrane proteins in the ER lumen: inositol-requiring protein 1 α (IRE1 α), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 α (ATF6 α), acting as sensors that physically bind with the glucose-regulated protein 78 (GRP78) to inhibit ER stress [18,19]. Emerging evidence has shown that ER stress is implicated in allergic airway diseases [20]. For example,

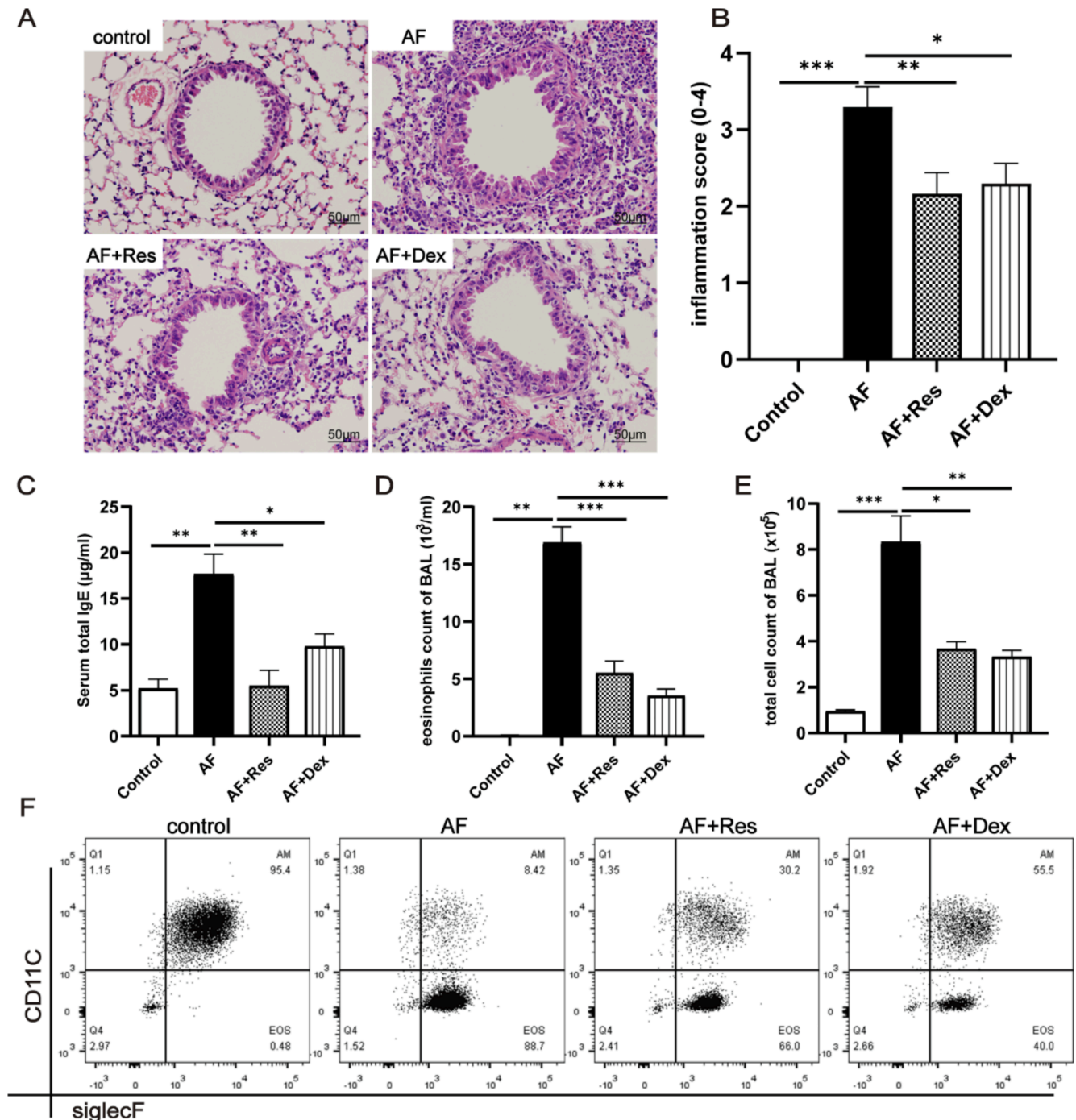


Fig. 1. Resveratrol attenuated Af-induced allergic lung inflammation. (A) Protocol for Af extract sensitization and exposure protocols. (B–C) Representative hematoxylin and eosin staining (magnification $\times 200$) (B) and the histological scores of lung inflammation (C). (D) Total serum IgE measured by ELISA. (E) The count of total cells and (F) eosinophils in BALF. (G) Representative flow cytometric dot plots of eosinophils (EOS) gated on CD45⁺Ly6G⁺CD11c⁺SiglecF⁺ cells in BALF. Data are mean $\pm$ SEM, $n = 5$ –8 for each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the Control or AF group.

ER stress inhibitor 4-phenylbutyric acid (4-PBA), or small interfering RNAs of ATF6 alleviated the lung inflammation induced by ovalbumin (OVA) [21] or house dust mite (HDM) [22]. Moreover, excessive ER stress ultimately leads to apoptosis, in which the PERK-ATF4 (activating transcription factor 4)-CHOP (C/EBP-homologous protein) pathway could initiate the apoptotic response [19,20,23]. In addition, airway epithelial apoptosis could be inhibited when ER stress was suppressed in asthmatic models [22]. Studies have reported that resveratrol could prevent tunicamycin-induced liver steatosis or protects against cadmium chloride-induced hippocampal neurotoxicity by alleviating ER stress [24,25]. At present, few studies have reported the effects resveratrol has on ER stress and apoptosis in fungus-related allergic lung disorders.

Thus, the present study is to elucidate the effects of salidroside on airway inflammation and apoptosis in Af-challenged allergic murine model and further explore its potential molecular mechanism about ER stress in vitro using a human bronchial epithelial cell line.

2. Methods and materials

2.1. Animals

Female wild-type BALB/c mice (6–8 weeks) were obtained from SLAC Laboratory Animals (Shanghai, China). All mice were housed under specific pathogen-free conditions and maintained on a 12 h light–dark rhythm in the Animal Center of Zhongshan Hospital. This study was approved by the Animal Care Committee of Fudan University Zhongshan Hospital (ID:2019–014) and the animal experimental protocols were performed following institutional guidelines.

2.2. Allergen sensitization and exposure

According to the protocol as previously described [26]. Briefly, mice were sensitized intraperitoneally with *Aspergillus fumigatus* extracts (Af, 20 µg per mouse; Greer Laboratories, Lenoir, North Carolina, USA) emulsified with aluminum (Imject Alum; Thermo Fisher Scientific, New York, USA) on days 0 and day 7, followed by intranasal inoculation with 25 µg Af extracts on day 14 and day 15. Resveratrol (50 mg/kg body weight; Res), dexamethasone (1 mg/kg body weight; Dex), or a vehicle was administered by a single intraperitoneal injection 1 h before each challenge. After the last challenge 24 h, all mice were sacrificed for lung tissue and blood (Fig. 1A). Bronchoalveolar lavage was performed twice with 1 ml of PBS via a tracheal catheter (80%–90% recovery rate). The control mice were sensitized and challenged with saline. Thus, these mice were randomly assigned to four groups (n = 5–8 mice per group) as follows: Control, AF, AF + Res, and AF + Dex.

2.3. Analysis of bronchoalveolar lavage fluid (BALF)

The supernatant after centrifugation was stored at – 80 °C until analysis and cell sediments were resuspended and determined by flow cytometric analysis as previously described [27,28]. Briefly, the recovered lavage cells were stained with anti-CD45, anti-Ly6G, anti-CD11c, and anti-Siglec-F (BioLegend or eBioscience, San Diego, CA, USA). Eosinophils were gated as CD45^{hi} Ly6G[–] CD11c[–] Siglec-F⁺ by flow cytometry (BD FACSAriaII). The total cell count of BALF was numerated and calculated by a hemocytometer.

2.4. Total IgE and cytokines measurement

Serum IgE level was measured with ELISA (Invitrogen, Thermo Fisher Scientific, Inc), and IL-4, IL-5, and IL-13 in BALF were detected by ELISA kit DuoSet (R&D system) according to the manufacturers' instructions.

2.5. RNA extraction and quantitative PCR analysis

Total RNA of lung tissue was extracted by Trizol reagents and then was reverse transcribed to cDNA using a Reverse transcript RNA kit (Toyobo, Shiga, Japan). The cDNA was quantified by real time-PCR using SYBR (Toyobo, Shiga, Japan), and analyzed with ABI 7500 RT-PCR system (Applied Biosystems, Foster, CA, United States). Except mouse β -actin primer was purchased from Sangon Biotech (Shanghai China), all other primers of genes were synthesized by Sangon Biotech, and their sequences were obtained from PrimerBank based on their gene ID (<https://pga.mgh.harvard.edu/primerbank/>). RNA relative expression levels were normalized to β -actin mRNA level.

2.6. Histopathology

The left upper lung lobe were isolated and fixed in 4% paraformaldehyde and embedded in paraffin. The lungs were cut into 5 µm sections, underwent dewaxing and rehydration, and were used for hematoxylin and eosin staining (HE). The degree of airway inflammation was evaluated by a double-blind method base on a scale in which 0 = no cells, 1 = a few cells, 2 = a ring of cells 1–2 cells deep, 3 = a ring of cells 3–4 cells deep, and 4 = more than 4 cells deep [11,13].

2.7. Western blot analysis

Cells and lung tissues were lysed in a lysis buffer containing protease and phosphatase inhibitor cocktail. BCA Assay was used to measure protein concentration. Lyses were boiled at 98°C for 5 min, and then resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes with 0.45 µm pore-size. The membranes were blocked for 1 h in 5% bovine serum albumin (BSA) solution and incubated overnight at 4 °C with primary antibodies as follows: anti-PERK (20582–1-AP; Proteintech), anti-ATF4 (sc-390063; Santa Cruz), anti-CHOP (2895 s; CST), anti-GRP78 (3183 s; CST), anti-caspase-3 (14220 t; CST), anti-caspase 7 (12827 t; CST) anti-cleaved caspase3 (9664 t; CST), anti-cleaved caspase7(8438 t; CST), anti-cleaved caspase 9 (52873 t; CST), anti-phospho-mTOR-ser2448 (AP0115; ABclonal), anti-phospho-Akt1-ser473 (AP0637; ABclonal), anti- β tubulin (10094–1-AP; Proteintech), and anti-GAPDH (10494–1-AP; Proteintech) at 1:1000 in primary antibody diluent. Followed by incubation with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h, the density was detected by chemiluminescence and was quantitatively analyzed using Image J 2.0

2.8. Immunofluorescence

According to the standard protocol, lung sections were first deparaffinized, followed by antigen retrieval using the citric acid antigen-retrieval solution. Slides were permeabilized, blocked with fetal bovine serum, washed, and then incubated overnight at 4°C with primary antibody anti-GRP78 antibody (GB11098; Servicebio, China) and anti-CHOP antibody (2895 s; CST). Next, the slides were washed and incubated with a secondary antibody for 1 h at room temperature. Then the slides were stained with DAPI and mounted after a wash. The tissue was visualized and photographed using Nikon Eclipse fluorescent microscope.

2.9. Terminal deoxynucleotidyl Transferase-Mediated nick end labeling assay (TUNEL)

As previously described, the apoptotic cells were detected in situ with TUNEL staining according to the manufacturer's instructions [15]. Images were captured by (Nikon, Tokyo, Japan). Apoptosis was quantified by determining the percentage of positively stained cells of DAPI positive cells in three random fields at 20 × magnification.

2.10. Cells and reagents

BEAS-2B cells were purchased from the Chinese Academy of Sciences (Shanghai, China). Beas-2B was cultured with DMEM/high glucose medium with 10% fetal bovine serum and 1% penicillin-streptomycin solution. *Aspergillus fumigatus* extracts (Greer Laboratories, Lenoir, North Carolina, USA) dissolved in saline and ultra-filtrated by 0.22 μ m sieve, and then was applied to experiments in vitro. Resveratrol (10 μ M; Selleck, Shanghai, China), LY294002 (10 μ M; Selleck, Shanghai, China), and 4-PBA (2.5 μ M; MCE, Shanghai, China) was pretreated 1 h before Af exposure, respectively.

2.11. Statistical analysis

All data were analyzed with GraphPad Prism version 8.0 software (GraphPad Software Inc, San Diego, CA, USA) and expressed as the mean $\pm$ SEM. The Student's *t*-test was used for the comparison of two groups. The one-way ANOVA followed by Tukey's post hoc testing with multiple comparison tests was used for three-plus groups. For all tests, *P*

value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Resveratrol inhibited Af-induced allergic lung inflammation.

Mice in the AF group showed evident lung inflammation, while decreased inflammatory cell infiltration was observed around the airway in the AF + Res group (Fig. 1B). Further quantification of lung inflammation degree consistently showed that resveratrol suppressed Af-induced lung inflammation ($P < 0.01$) (Fig. 1C). In addition, the level of serum IgE was attenuated in the AF + Res group relative to that in the AF group ($P < 0.01$) (Fig. 1D). Total cell counts of BAL increased in the AF group ($P < 0.001$), especially the dominant inflammatory cells eosinophils, whereas diminished significantly in the AF + Res group ($P < 0.05$) (Fig. 1 E-F). Flow cytometry analysis was used to distinguish eosinophils (CD11c⁺SiglecF⁺) gated on cells (CD45⁺Ly6G⁺) in BALF (Fig. 1G). Taken together, these findings indicated that resveratrol plays an anti-inflammatory and anti-allergic role in Af-exposed lung

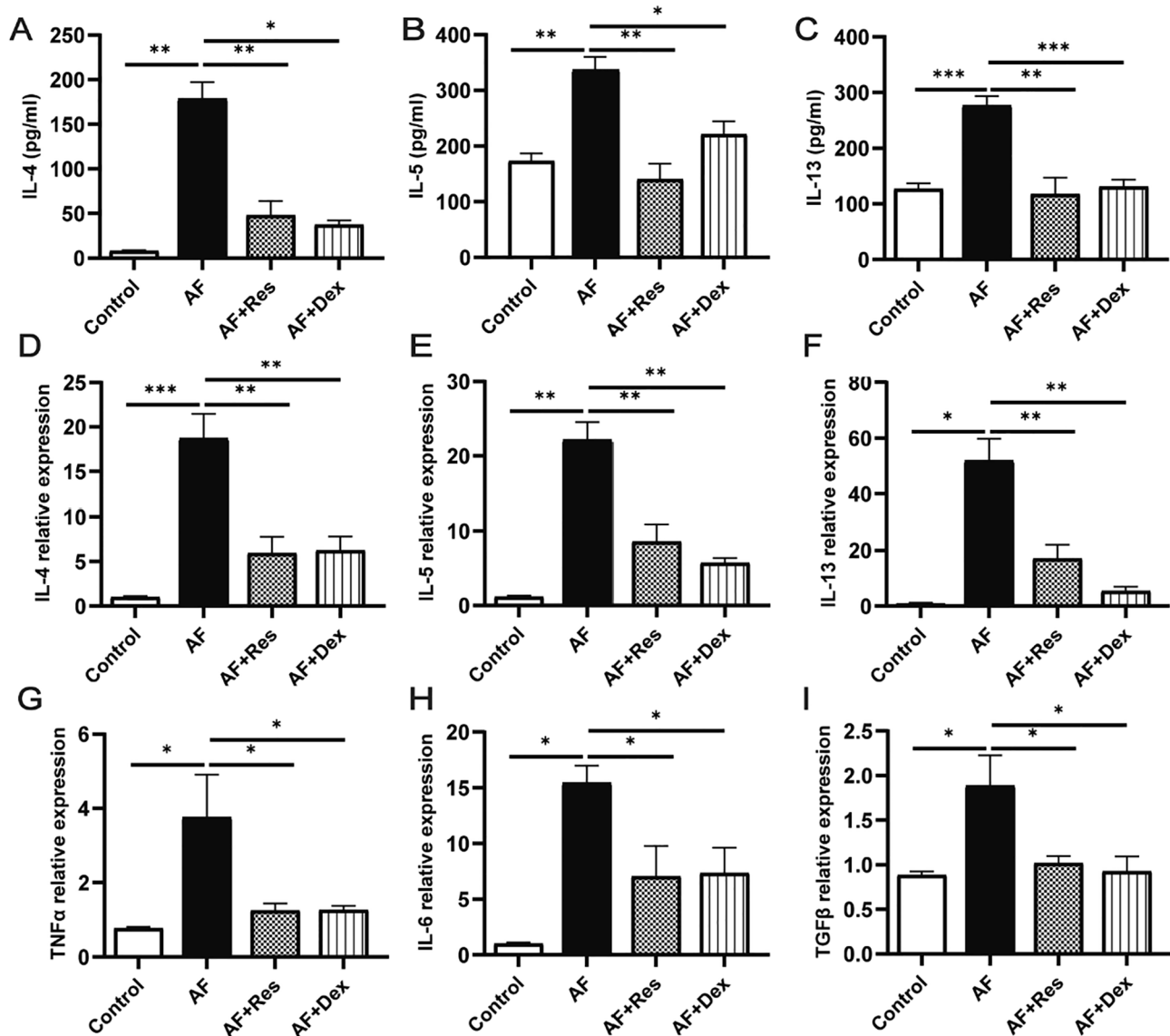


Fig. 2. Resveratrol decreased inflammatory cytokines levels in Af-induced allergic lung inflammation. (A-C) The levels of IL-4 (A), IL-5 (B), and IL-13 (C) in BALF measured by ELISA. (D-F) The mRNA expressions of IL-4 (D), IL-5 (E), and IL-13 (F) in lung tissues quantified by quantitative PCR. (G-H) The mRNA expressions of TNF- α (G), IL-6 (F), and TGF- β (F) in lung tissues quantified by quantitative PCR. Data are mean $\pm$ SEM, *n* = 5–8 for each group. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with the Control or AF group.

inflammation.

3.2. Resveratrol decreased inflammatory cytokines production in Af-induced allergic lung inflammation.

Previous studies have reported that resveratrol could inhibit the production of cytokines such as Th2 cytokines, TNF- α , TGF- β in OVA or Endoplasmic reticulum stress mediates hous-induced asthmatic models [10–12,14], so we assessed expressions of these cytokines in the four groups. As seen in Fig. 2A–2C, protein levels of IL-4, IL-5, and IL-13 in BALF were significantly upregulated in mice of AF group, while expressions of these Th2 cytokines were reduced in the AF + Res group ($P < 0.01$). RT-PCR of lung tissues also proved that mRNA levels of IL-4, IL-5, and IL-13 were downregulated remarkably by resveratrol or

dexamethasone ($P < 0.01$) (Fig. 2D–2F). Besides, TNF α , IL-6, and TGF- β demonstrated a decrease in AF + Res and AF + Dex group ($P < 0.05$) compared with those of the AF group (Fig. 2G–I).

3.3. Resveratrol inhibited Af-induced lung endoplasmic reticulum stress.

It was indicated that ER stress regulated by PI3K δ might play a crucial role in the development of Af-induced severe allergic inflammation [29]. Thereby, the study next investigated whether resveratrol could attenuate lung ER stress induced by Af. Representative double immunofluorescent staining showed that resveratrol inhibited lung expressions of GRP78 and CHOP, both of which were increased in the AF group compared to the control group (Fig. 3A). The mRNA expressions of GRP78, PERK, ATF4, and CHOP in the lung tissues were lower in the

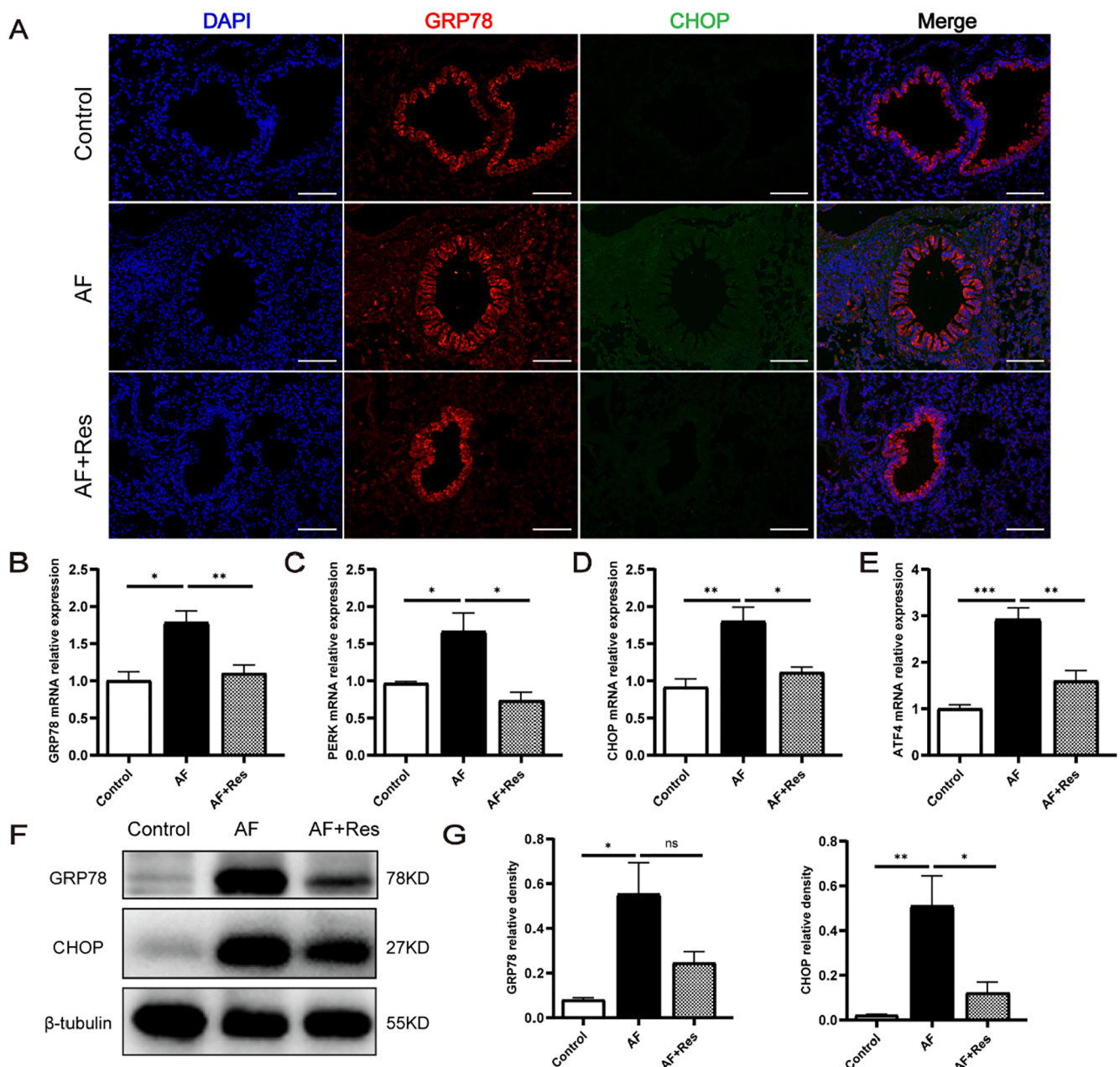


Fig. 3. Resveratrol inhibited endoplasmic reticulum stress in the lungs of Af-exposed mice. (A) Representative immunofluorescence photomicrographs of GRP78 and CHOP in lungs (magnification $\times 200$). (B–E) Relative lung mRNA expressions of GRP78 (B), PERK (C), ATF4 (D), and CHOP (E) quantified by qPCR. (F) Representative western blots of GRP78 and CHOP and (G) densitometric analyses normalized to β -tubulin. Data are mean $\pm$ SEM. $n = 4$ –6 for each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the Control or AF group. Bars indicating 100 μ m.

presence of resveratrol and higher in the absence ($P < 0.05$) (Fig. 3B-E). Furthermore, western blot analysis (Fig. 3F-G) also confirmed that ER stress was inhibited by resveratrol due to the lower protein levels of GRP78 and CHOP in the AF + Res group relative to the AF group ($P < 0.05$).

3.4. Resveratrol inhibited lung apoptosis in Af-exposed allergic lung inflammation.

Excessive ER stress could mediate cell death by PERK/ATF4/CHOP pathway, and CHOP was able to downregulate the expression of anti-apoptotic proteins and upregulate the Bcl-2 proapoptotic protein family [18,19]. To study the effects of resveratrol on apoptosis, we tested the protein levels of executive caspase proteins and cleaved PARP in the lung tissues of mice. As Fig. 4A-G shown, cleaved caspase-3, and cleaved caspase-7 were all increased in Af-exposed mice ($P < 0.01$) but suppressed by resveratrol ($P < 0.05$). Moreover, TUNEL staining further confirmed that a significantly increased percentage of apoptotic cells in lungs of Af-challenged mice while decreased in the AF + Res group ($P < 0.05$) (Fig. 4F-G).

3.5. Resveratrol inhibited ER stress and apoptosis in airway epithelial cells following Af stimulation.

Airway epithelial cells act as the first line of defense against

environmental factors such as pathogens and allergens, involving in the regulation of airway inflammatory status, structure, and function in normal and diseased airways [30,31]. Previously, it was reported that the allergen HDM could induce apoptosis via activating ER stress in airway epithelial cells [22]. We next test the expressions of GRP78, PERK, ATF4, and CHOP in vitro to evaluate ER stress in Af-exposed human bronchial epithelial cells. As shown in Fig. 5A-D, the protein levels of GRP78, PERK, ATF4, and CHOP were increased in a dosage-dependent and time-dependent manner in Af-exposed BEAS-2B cells ($P < 0.05$). Pretreatment BEAS-2B with resveratrol 1 h followed by Af stimulation significantly reduced level of ER stress. (Fig. 5E-F, $P < 0.05$).

3.6. Resveratrol inhibited ER stress via Akt/mTOR and ER stress mediated apoptosis in Af-exposed BEAS-2B cells

Resveratrol could play anti-inflammatory effects via regulating PI3K/Akt/mTOR [16]. Our results showed that Af extract activated the Akt/mTOR pathway in a dosage-dependent manner in vitro ($P < 0.05$) (Fig. 6A-B). Then, we examined the relationship between Akt/mTOR pathway and resveratrol in BEAS-2B cells with Af stimulation. Protein expressions of p-AKT and p-mTOR were remarkably reduced by resveratrol ($P < 0.05$) (Fig. 6C-D). Using LY294002 (pharmacologic inhibitor of Akt) suppressed the levels of GRP78 and CHOP in Af-exposed BEAS-2B ($P < 0.05$) (Fig. 6E-F). Lastly, we hypothesized that activated ER stress mediated Af-induced apoptosis in BEAS-2B cells.

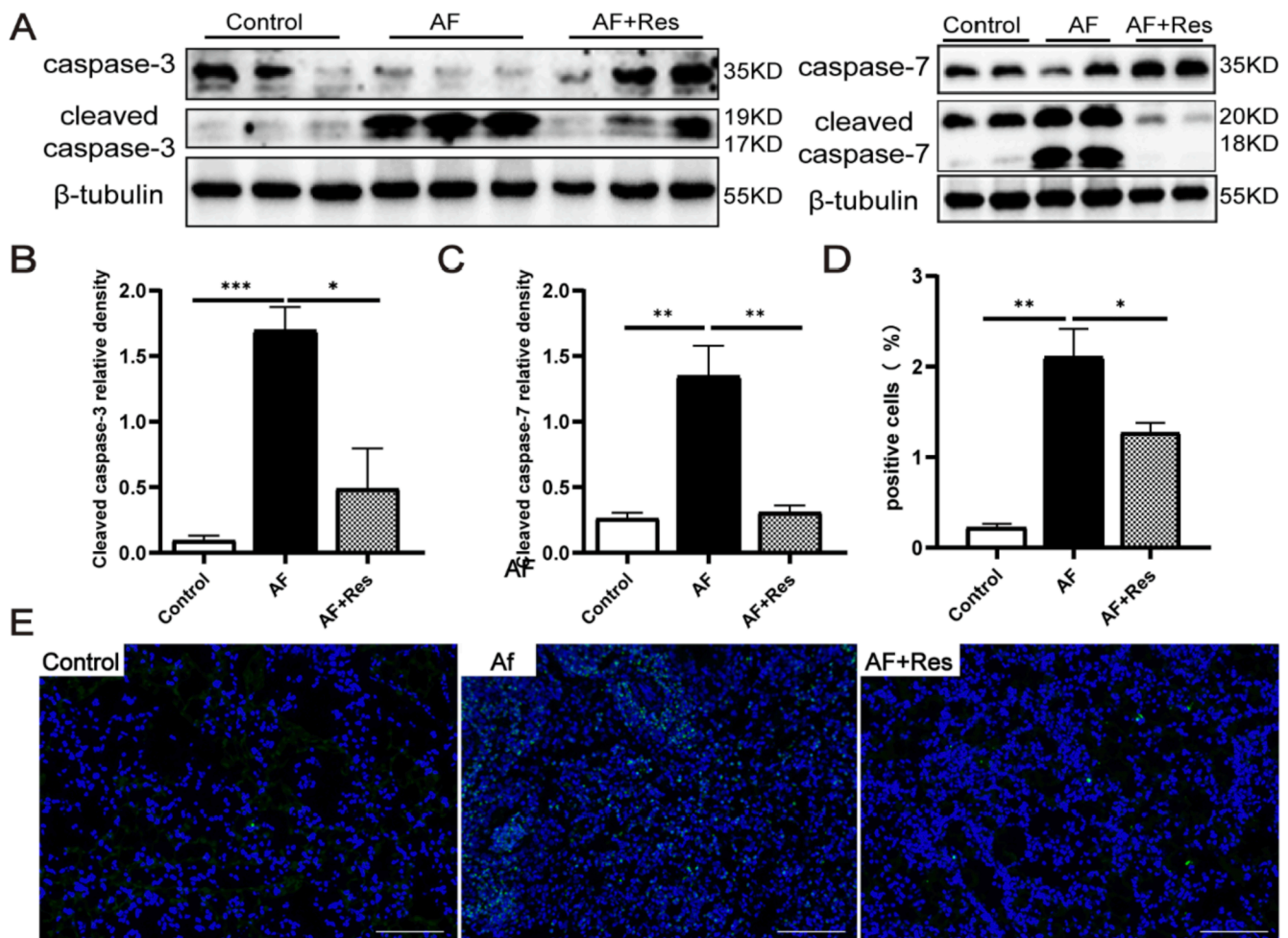


Fig. 4. Resveratrol inhibited apoptosis in lungs of Af-exposed mice. (A) Representative western blots of cleaved caspase-3 and cleaved caspase-7 and (B-C) densitometric analyses normalized to β-tubulin. (E) Representative lung images of TUNEL assay (magnification $\times 200$) and (D) quantification of TUNEL-positive cells (green). Data are mean $\pm$ SEM, $n = 4-6$ for each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the Control or AF group. Bars indicating 100 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

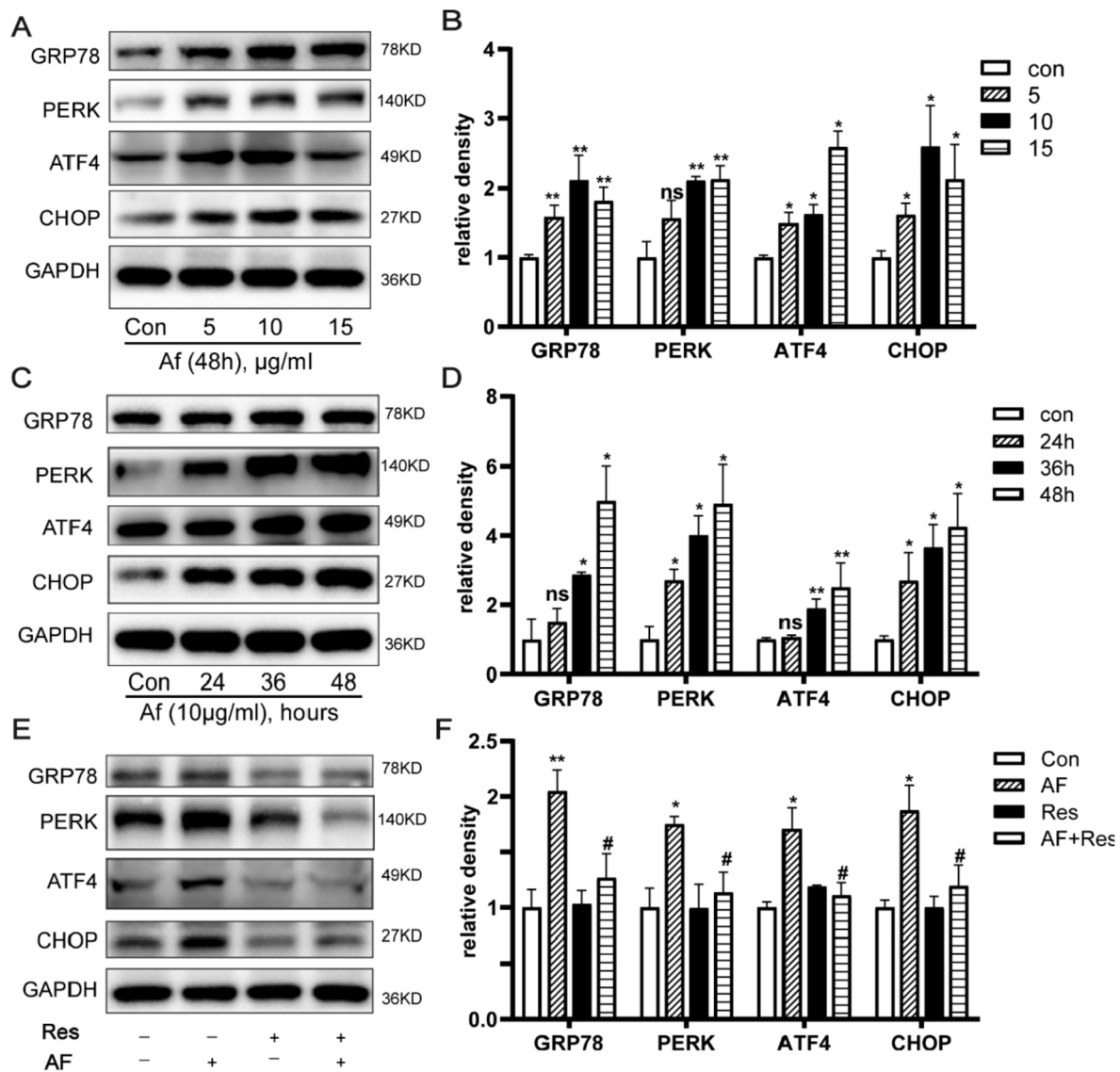


Fig. 5. Resveratrol inhibited ER stress in Af-exposed airway epithelial cells. Representative western blots and densitometric analyses of GRP78, PERK, ATF4, and CHOP levels in Af-exposed BEAS-2B (A-B) stimulated with different dosages and (C-D) treated at different time points. (E-F) Protein levels of GRP78, PERK, ATF4, and CHOP were measured by western blots in BEAS-2B cells that were pretreated with resveratrol (10 µM) 1 h and then subjected to Af stimulation (10 µg/ml) for 48 h. Data are mean $\pm$ SEM, $n = 3$ for each group and $N = 3$ independent experiments. * $P < 0.05$, ** $P < 0.01$, and ns no significance, compared with the Control group; # $P < 0.05$, compared with AF.

Pretreatment with 4-PBA inhibited the levels of GRP78 and CHOP as well as the cleaved caspase-7 and cleaved caspase-9 (Fig. 6G-H, $P < 0.05$), confirming the hypothesis that apoptosis was mediated by ER stress in Af-exposed BEAS-2B cells.

4. Discussion

In the current study, we firstly showed that resveratrol treatment significantly alleviated bronchial inflammation, reduced levels of Th2 type and other inflammatory cytokines, and suppressed effectively ER stress and apoptosis in the Af-induced allergic asthmatic murine model (Fig. 7). Additionally, resveratrol inhibited Af-induced ER stress in human bronchial epithelial cells, and Akt/mTOR pathway might be involved in the regulation of ER stress (Fig. 7). These findings demonstrate that resveratrol was beneficial for Af-exposed allergic lung inflammation, which may be associated with inhibition of ER stress and apoptosis via the Akt/mTOR pathway.

Previous studies have demonstrated resveratrol has the anti-allergic,

anti-inflammatory, and anti-airway remodeling effects in asthma [10–14]. Resveratrol could attenuate Th2-mediated immune response by downregulating miR-34a mediated expression of FOXP3 in OVA-induced mouse asthma [10]. Resveratrol could also reduce inflammatory cytokines such as TGF- β , IL-6, IL-17, and TNF- α in BALF in HDM-challenged mice, indicating the suppression of inflammatory response [11,32]. In addition, TGF- β 1/Smad signaling and TGF- β level were reported to reverse airway remodeling [11,12]. Consistent with the findings of the studies above, our results firstly showed that resveratrol could also ameliorate Af-induced allergic lung inflammation and decrease not only Th2 cytokines but the cytokines including TGF- β , IL-6, and TNF- α .

Emerging evidence has suggested that ER stress plays a critical role in the pathogenesis of allergic lung disorder [18,23]. Importantly, it had been proved that ER stress was increased in Af-induced steroid-resistant allergic lung inflammation, and the PI3K δ /Akt pathway was involved in the regulation of ER stress [29]. Our study consistently found that lung ER stress was increased significantly in Af-exposed mice and Af-

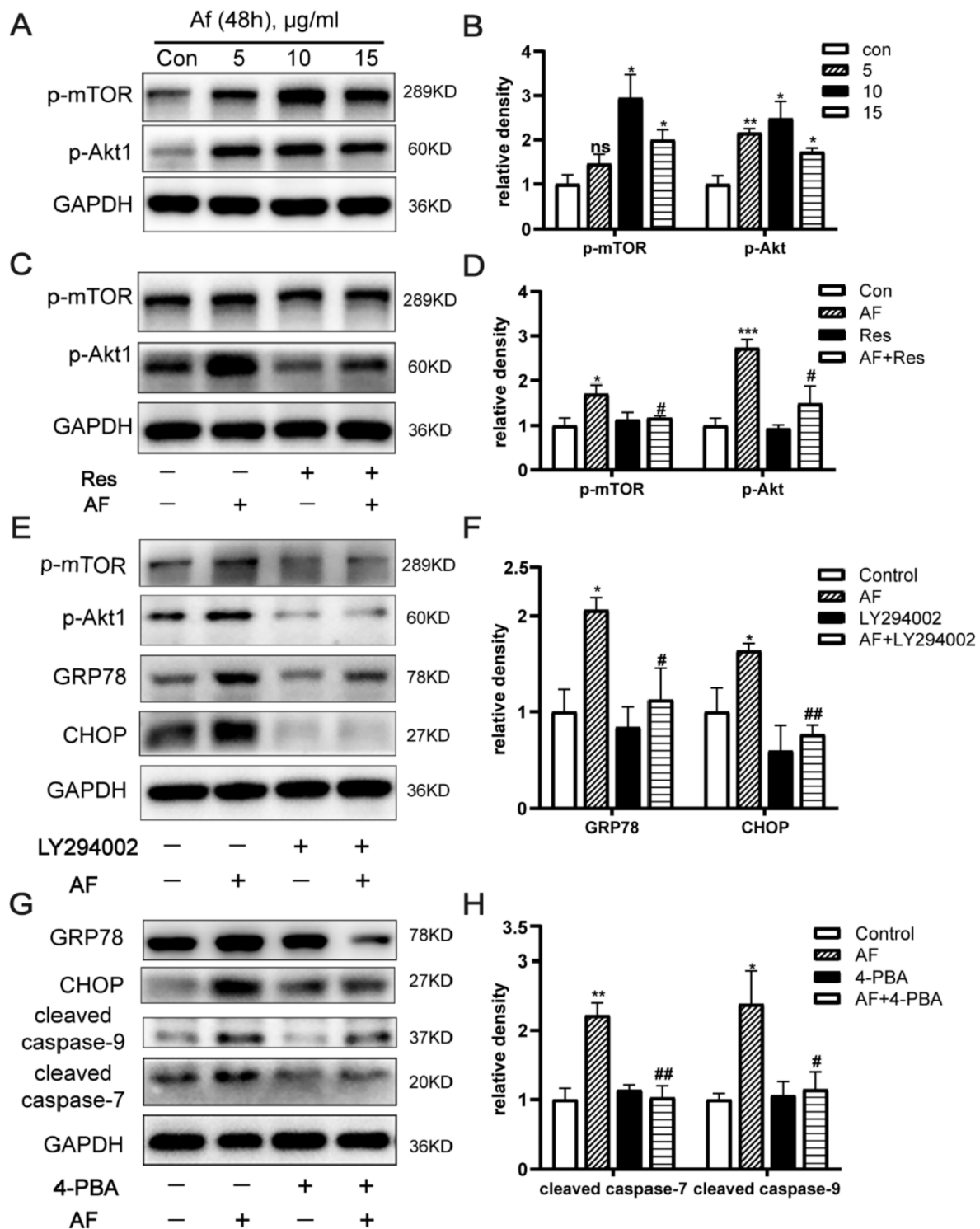


Fig. 6. Resveratrol inhibited Akt/mTOR pathway to regulate ER stress and ER stress mediated apoptosis in Af-exposed BEAS-2B cells. Representative western blotting of p-Akt1 and p-mTOR and analyses normalized to GAPDH in (A-B) BEAS-2B cells exposed with different dosages of Af at 48 h, and (C-D) BEAS-2B cells pretreated with resveratrol (10 µM) 1 h and followed by Af stimulation (10 µg/ml) for 48 h. (E-F) Western blots and quantification of GRP78 and CHOP in Af-exposed cells that were pretreated with LY294002 (10 µM) 1 h and then subjected to Af extract (10 µg/ml, 48 h). (G-H) Western blot analysis of cleaved caspase-7 and cleaved caspase-9 in BEAS-2B cells pretreatment with 4-PBA (2.5 µM) 1 h following Af stimulation (10 µg/ml, 48 h). Data are mean $\pm$ SEM, n = 3 for each group and N = 3 independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001, compared with the Control group; # P < 0.05, ## P < 0.01, compared with AF.

stimulated human bronchial epithelial cells. While previous studies demonstrated that resveratrol exerted protective effects in tunicamycin stimulated mice [24] or HT22 cells [33] and prevented the progression of hepatic steatosis in high-fat diet-fed rats through alleviating ER stress [24,34]. In addition, inhibition ER stress with chemical chaperones such

as 4-PBA or tauroursodeoxycholic acid reduced the allergic lung inflammation [29,35]. Thus, we explored the effects resveratrol had on ER stress in Af-challenged mice and Af-exposed airway epithelial cells. Our findings firstly showed that resveratrol suppressed the Af-induced ER stress both in vivo and in vitro, suggesting that the potential

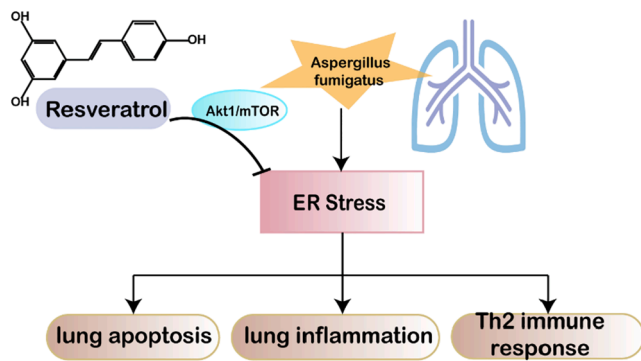


Fig. 7. Overview on the effects and mechanisms for the involvement of resveratrol in *Aspergillus fumigatus* (Af)-exposed allergic lung inflammation. ER stress: endoplasmic reticulum (ER) stress.

therapeutic role resveratrol played on Af-induced allergic lung inflammation might be associated with the suppressed ER stress.

When overwhelmed proteins in ER exceed the folding capacity of ER, damaged cells were further eliminated by apoptosis [18,19]. A study has revealed that upregulation of ATF4 and CHOP promoted the epithelial cell apoptosis in cigarette smoke extract stimulated human bronchial epithelial (HBE) cells [36], implicating that ATF4/CHOP pathway was involved in the crosstalk between ER stress and apoptosis [19]. Moreover, CHOP can activate pro-apoptotic proteins like BIM, death receptor 5 (DR5), and reduced Bcl-2 anti-apoptotic protein [37]. Our results have displayed the Af stimulation induced the ER stress and apoptosis both in vivo and increased the ER stress in vitro, whose suppression inhibited the apoptosis in human bronchial cells. Thus, ER stress may be a potential target for lung epithelial apoptosis induced by Af [22,36].

However, the mechanisms behind resveratrol inhibited ER stress and apoptosis in Af-induced allergic lung inflammation need to be further studied. The effects of resveratrol on the Akt/mTOR pathway have been studied in different contexts [16]. It was reported that resveratrol attenuated bronchial asthma by restoring lung INPP4A level which in turn inhibited Akt kinase activity and Akt phosphorylation [38]. Additionally, resveratrol alleviated IL-33-mediated mast cell activation and the intestinal injury of irradiated rats through the PI3K/Akt pathway [39,40]. Furthermore, in Af-exposed or OVA/LPS-induced allergic lung inflammation, the PI3K δ /Akt pathway regulated ER stress via activation of NF- κ B [17,29]. Here our findings confirmed that resveratrol inhibited ER stress as well as downregulated Akt/mTOR pathway which was activated in Af-exposed human bronchial cells, and LY292004 could alleviate ER stress significantly through inhibiting Akt, indicating that resveratrol might regulate ER stress via Akt/mTOR pathway in Af-induced allergic lung disorders.

However, the study had some limitations. First, the original forms of proteins including Akt, mTOR, and caspases were not detected although their active forms could represent the activation degree to some extent. Second, we did not employ ER stress inducer or Akt/mTOR agonist to further prove that resveratrol inhibited apoptosis via suppressing ER stress and inhibited ER stress through Akt/mTOR pathway. Third, there are no defined standards of ABPA animal models. The model we established only represented the acute Af-exposed allergic lung inflammation. Lastly, only one dosage of resveratrol was used in vivo and in vitro. Thus, long-term fungus-exposed models and dosage-response studies are required to explore for further study.

Our study indicated that *resveratrol* treatment suppressed the Af-induced allergic lung inflammation and inhibited Af-induced bronchial epithelial cells apoptosis through alleviating ER stress via Akt/mTOR pathway. To the best of our knowledge, we firstly report a novel finding that resveratrol has protective effects on fungus-induced allergic lung disorders, hoping to provide further evidence for the treatment of ABPA.

CRediT authorship contribution statement

Sijiao Wang: Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Linjing Gong:** Investigation, Methodology, Data curation, Writing – original draft. **Yuqing Mo:** Methodology, Data curation, Validation. **Jun Zhang:** Methodology, Validation. **Zhilong Jiang:** Validation, Writing – review & editing. **Zhengan Tian:** Funding acquisition, Writing – review & editing. **Changzhou Shao:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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