International Journal of Chronic Obstructive Pulmonary Disease

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Cholesterol Accumulation Enhances Cigarette Smoke-Induced Airway Epithelial Inflammation

Du Jing[1,2], Jin-Kang Yu[1], Hai-Pin Chen[1], Ling-Ling Dong[1], Wen Li[1], Zhou-Yang Li[1], Jie-Sen Zhou[1]

[1]Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, People's Republic of China; [2]Department of Respiratory and Critical Care Medicine, Yuyao People's Hospital of Zhejiang Province, Ningbo, People's Republic of China

Correspondence: Jie-Sen Zhou; Zhou-Yang Li, Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, 310009, People's Republic of China, Email jason.627@zju.edu.cn; lizhouyang@zju.edu.cn

Video Abstract

Point your SmartPhone at the code above. If you have a QR code reader the video abstract will appear. Or use: https://youtu.be/EYe05ZynRxI.

Background: Statins, commonly used to lower cholesterol, have been shown to have anti-inflammatory effects in respiratory disease models. Disorders of cholesterol metabolism can cause damage to cells and tissues, and further lead to the development of a variety of diseases. However, the role of cholesterol metabolism in cigarette smoke-induced airway epithelial inflammation is unclear. The present study aims to explore this question.

Methods: Human bronchial epithelial cells (HBEs) were stimulated with cigarette smoke extract (CSE) and mice were exposed to CS as models. The expression of cholesterol content and cholesterol metabolism-related molecules such as Sterol Regulatory Element-Binding Protein 2 (SREBP2), 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR), ATP Binding Cassette Transporter A1 (ABCA1), and ATP Binding Cassette Transporter G1 (ABCG1) were detected by cholesterol assay kits and immunohistochemistry (IHC) in vivo, and were detected by cholesterol assay kits, Western blot (WB), and quantitative real-time polymerase chain reaction (Q-PCR) in vitro. Cholesterol metabolism molecules related siRNAs, inhibitors or agonists were used to intervene the Cholesterol levels in HBE. The mRNA level and protein level of interleukin IL-6 and IL-8 were detected by RT-qPCR and enzyme-linked immunosorbent assay (ELISA). Cellular reactive oxygen species (ROS) levels were detected by reactive oxygen species assay kits.

Results: We found that cigarette smoke exposure inhibited cholesterol efflux by decreasing the expression of ABCA1, thereby increasing cholesterol accumulation in airway epithelial cells, which promotes the production of reactive oxygen species and promotes the secretion of inflammatory cytokines, ultimately aggravating cigarette smoke-induced airway inflammation. Reducing intracellular cholesterol content by inhibiting intracellular synthesis and promoting increased efflux can reduce the cigarette smoke-induced airway epithelial inflammatory factors secretion.

Conclusion: In conclusion, cholesterol accumulation plays an important role in cigarette smoke-induced airway inflammation and may provide new targets in the treatment of this disease in the future.

Keywords: airway epithelial cell, cholesterol metabolism, cigarette smoke, inflammation

Introduction

Chronic obstructive pulmonary disease (COPD) is a common and deadly disease worldwide. According to the 2024 Global Initiative for Chronic Obstructive Lung Disease (COPD) guidelines, COPD ranks among the top three causes of death globally, claiming over 3 million lives in 2012, which represents 6% of total global deaths.[1] In China, COPD ranks third as a cause of death,[2] and its prevalence is expected to increase due to factors such as aging populations, smoking, and air pollution. However, the exact mechanisms causing COPD remain unclear, leading to ongoing debates regarding its diagnostic criteria and treatment strategies.[3] Therefore, investigating the fundamental mechanisms of COPD and exploring potential therapeutic targets are crucial for effectively managing and preventing this disease.

Airway epithelial cells are the first cell type in the respiratory system to come into contact with the external environment.[4] These cells not only serve as a protective barrier but also secrete various cytokines and growth factors.

Received: 12 October 2024
Accepted: 3 February 2025
Published: 21 February 2025

Smoking is a common risk factor for COPD. Cigarette smoke activates epithelial cells and innate immune cells, inducing the release of pro-inflammatory cytokines and chemokines from airway epithelial cells and alveolar macrophages.[5] Therefore, studying the role of airway epithelial cells in the pathogenesis of COPD is crucial for understanding the disease mechanisms and for developing preventive and therapeutic strategies. Besides being a risk factor for COPD, cigarette smoke also leads to dyslipidemia and reduces the levels of high-density lipoprotein (HDL), significantly increasing the incidence and mortality of cardiovascular diseases.[6]

Statins, which are drugs that lower cholesterol levels mainly by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), are commonly used to treat atherosclerosis and have now been shown to have anti-inflammatory effects in respiratory diseases.[7,8] Cholesterol is widely present in animals; in addition to being a component of cell membranes, it is a precursor for the synthesis of bile acids, vitamin D, and various steroid hormones, making it an essential component of animal tissues and cells.[9] Therefore, dysregulation of cholesterol metabolism can cause cellular and tissue damage, leading to the development and progression of various diseases.[10] Cholesterol metabolism mainly involves four processes: synthesis, uptake, efflux, and esterification. The cholesterol biosynthesis pathway includes three key molecules: sterol regulatory element-binding protein 2 (SREBP2) and two rate-limiting enzymes, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and squalene monooxygenase.[11] When intracellular cholesterol is excessive, it leads to the formation of specific sterols, thereby activating the liver X receptor (LXR)-retinoid X receptor (RXR) heterodimeric transcription factor, which promotes cholesterol efflux from cells by upregulating the expression of ABCA1 and ABCG1.[12]

An increasing number of studies have shown that cholesterol is associated with lung inflammation and damage in patients.[11] Recent studies have shown that external cholesterol may alter the sensitivity of airway epithelial inflammation to CSE by modulating the STARD3-MFN2 pathway, cholesterol redistribution, changes in cholesterol transport and accumulation, lipid transport regulator activity, and disruption of mitochondrial function and dynamics.[13] This study suggests that cholesterol excess is associated with chronic obstructive pulmonary disease and airway epithelial-driven inflammation. However, the phenotype of local cholesterol metabolism in cigarette smoke-induced airway inflammatory diseases and its potential role and mechanisms in regulating associated inflammatory responses remain unclear. This study aims to investigate the changes in cholesterol levels and the phenotypic alterations of metabolism-related molecules in airway epithelial cells induced by cigarette smoke. Furthermore, it seeks to elucidate the potential mechanisms by which cholesterol regulates cigarette smoke-induced airway inflammation, thereby providing new insights and therapeutic targets for the prevention and control of the disease.

Materials and Methods

Animal Models and Immunohistochemistry

Wild-type C57BL/6J mice (6–8 weeks old) were purchased from Shanghai Slack Laboratory Animal Co., LTD. (Shanghai, China), and housed in a temperature- and humidity-controlled room in Laboratory Animal Center of Zhejiang University. All mice were housed in a specific pathogen-free facility. Ethical approval for all experimental procedures was granted by the Zhejiang University Medical Laboratory Animal Care and Use Committee and the Ethics Committee for Animal Studies at Zhejiang University. All operations adhere to the "Basic Requirements for Laboratory Animal Welfare and Ethics in Experiments" (GB/T 35892-2018). For the whole body smoking exposure model, mice were exposed to the smoke of 10 cigarettes via a machine (TE-10, Teague Enterprises) as previously described.[14] The mice were exposed 2 h/day, 5 days/wk for 1, 3, and 6 months. Control mice were exposed to normal air. 24 hours after the final exposure, mice were anesthetized with 2% pentobarbital sodium via intraperitoneal injection. Blood was collected from the right ventricle. The right lung hilum was ligated, and the right lung lobes were tied off and associated blood vessels ligated. The trachea was exposed and cannulated, and 200 μL of 4% formaldehyde was instilled into the left lung for in situ fixation. The trachea, heart, and left lung were excised and placed in 10 mL of 4% formaldehyde for further fixation. The samples were prepared for immunohistochemistry staining of SREBP-2 according to the manufacturer's instructions. Image quantitative analysis was performed showing the ratio of SREBP-2-positive to total epithelial cells.

Cell Culture and Agents

Human bronchial epithelial (HBE) cells were purchased from American Type Culture Collection (ATCC, CRL-2741), and were maintained in RPMI 1640 with 10% FBS, 50 U/mL penicillin, and 50 U/mL streptomycin. HBE cells were treated with 1.5% CSE in the presence of Lovastatin (S2061, Selleck, USA), GW3965 (S2630, Selleck, USA) or DMSO (67-68-5, Sigma, USA) for 30 minutes. 2 hours with 1.5% CSE in the absence or presence of MCD (128446-36-6, Sigma, USA). Treatment with 1.5% CSE for 6 hours in the absence or presence of Water-soluble cholesterol (C4951, Sigma, USA).

CSE Preparation and Treatment

Standard research cigarettes (Baisha Cigarette, products from China Tobacco Industrial Co., LTD., China) were used in the study. CSE was prepared as described previously.[14] In brief, smoke of 1 cigarette was bubbled slowly through 10 mL of RPMI 1640, which was considered as 100% CSE solution, and was then sterilized and stored at 80°C. HBE cells were treated with 1.5% CSE for 6 hours for cholesterol content testing, and IL-6 and IL-8 mRNA or protein levels are detected for 12 h. Treatment with 5% CSE for 12 hours for apoptotic levels testing.

siRNA Transfection in vitro

When HBE cells reached 30–50% confluence, they were transfected with SREBP2-siRNA (sc-36559, SantaCruzBiotechnology, USA), HMGCR-siRNA (sc-44851, SantaCruzBiotechnology, USA), ABCA1-siRNA (sc-41137, SantaCruzBiotechnology, USA) or Control-siRNA (sc-37007, SantaCruzBiotechnology, USA) at 10 nM. Fresh medium was added 30–60 minutes before transfection. Transfection mixture (Transfection reagent and Transfection Buffer, #SL100568, Genmute, USA) was added to the medium and mixed gently. The medium was changed 5 hours post-transfection. Samples were collected 24 hours post-transfection for RNA analysis and 48 hours for protein analysis.

Plasmid Transfection in vitro

When HBE cells reached 90% confluence, Transfection of LXRa overexpression plasmid (Designed by GeneCopoeia, USA) was conducted by Lipofectamine 3000 (L3000001, ThermoFisher Scientific, MA, USA). LXRa overexpression plasmid was diluted in 50 μL serum-free medium, and the transfection reagent was diluted in 50 μL serum-free medium. After a 5-minute incubation at room temperature, the mixtures were combined and incubated for 30 minutes. The transfection mixture was added to cells and mixed gently. Medium was changed 4–6 hours post-transfection. Gene expression was analyzed 18–48 hours post-transfection.

Cholesterol Assay

The cholesterol contents of HBE cells was detected by Amplex Red cholesterol assay kit (A12216, Thermo Scientific, USA). The treated samples were added to black 96-well plates and mixed with working reagent (5 mL: 4.82 mL 1x buffer, 75 μL Reagent A, 50 μL HRP, 50 μL cholesterol oxidase, 5 μL cholesterol esterase). The mixture was incubated at 37°C for 30 min in the dark. Fluorescence was measured at 540 nm excitation and 590 nm emission. Background fluorescence was subtracted, and cholesterol content was calculated from a standard curve. The final cholesterol levels were normalized to total protein content (BCA Assay, 23235, Thermo Scientific, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Trizol (#15596026, Invitrogen, CA, USA) was used to extract total RNA. Then, mRNA was reverse-transcribed to cDNA by PrimeScript RT Master Mix (RR037A, Takara, Japan). The real-time PCR was performed by TB Green Premix Ex Taq (RR420A, Takara, Japan). The PCR primer sequences were obtained from PrimerBank (https://pga.mgh.harvard.edu/primerbank/) and listed in Table 1.

Western Blot Assay

After treatment, HBE cells were lysed in RIPA buffer (150 mm NaCl, 50 mm Tris pH 8.0, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS, supplemented with protease and phosphatase inhibitors). Proteins in lysates of cells were separated by SDS-PAGE and immunoblotted using antibodies against the following proteins: SREBP2 (ab30682,

Table 1 The PCR primer names and primer sequences

Primer	Primer Sequence	
Human ACTB	Forward:	CATGTACGTTGCTATCCAGGC
	Reverse:	CTCCTTAATGTCACGCACGAT
Human SREBP2	Forward:	CTGCAACAACAGACGGTAATGA
	Reverse:	CCATTGGCCGTTTGTGTCAG
Human HMGCR	Forward:	CGTGGAATGGCAATTTTAGGTCC
	Reverse:	ATTTCAAGCTGACGTACCCCT
Human ABCA1	Forward:	ACCCACCCTATGAACAACATGA
	Reverse:	GAGTCGGGTAACGGAAACAGG
Human ABCG1	Forward:	GGGGTCGCTCCATCATTTG
	Reverse:	TTCCCCGGTACACACATTGTC
Human IL6	Forward:	CCTGAACCTTCCAAAGATGGC
	Reverse:	TTCACCAGGCAAGTCTCCTCA
Human IL8	Forward:	TTTTGCCAAGGAGTGCTAAAGA
	Reverse:	AACCCTCTGCACCCAGTTTTC
Human LXRa	Forward:	CCTTCAGAACCCACAGAGATCC
	Reverse:	ACGCTGCATAGCTCGTTCC

Abbreviations: ACTB, β-Actin; SREBP2, Sterol Regulatory Element-Binding Protein 2; HMGCR, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; ABCA1, ATP Binding Cassette Transporter A1; ABCG1, ATP Binding Cassette Transporter G1; IL6, Interleukin 6; IL8, Interleukin 8; LXRa, Liver X Receptor Alpha.

1:1000, Abcam, USA), HMGCR (ab242315, 1:1000, Abcam, USA), ABCA1 (NB400-105, 1:500, Novus Biologicals, USA), ABCG1 (ab52617, 1:1000, Abcam, USA), LXRa (ab176323, 1:1000, Abcam, USA), β-actin (sc-47778, 1:1000, Santa Cruz Biotechnology, USA), LaminA/C (sc-7292, 1:1000, Santa Cruz Biotechnology, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

Using a Human IL-6 ELISA Kit (RDR-IL6-Hu-96T, Dakowei, China) and IL-8 ELISA Kit (RDR-IL8-Hu-96T, Dakowei, China), cell culture supernatants were collected in 1.5 mL EP tubes, centrifuged at 3000 g for 20 min at 4°C, and diluted as needed. 50 μL of anti-IL6 antibody and 50 μL of diluted samples were added to each well. Plates were incubated at room temperature for 1 hour, washed 3 times with 1x Wash Buffer, and 100 μL HRP was added to each well. After a 20-minute incubation, wells were washed, 100 μL of substrate solution was added, and plates were incubated in the dark for 5–30 minutes. The reaction was stopped with 100 μL of stop solution, and OD was measured at 450 nm with a 570 nm correction. Protein concentration was calculated from a standard curve.

ROS Detection in Cells

DCFH-DA (S0033S, BeyotimeBiotechnology, China) was diluted 1:1000 in serum-free medium to a final concentration of 10 μmol/L. Cells were incubated with the diluted DCFH-DA at 37°C for 20–30 minutes in the dark. After washing with PBS, cells were digested with trypsin and neutralized with serum-containing medium. Cells were collected into flow cytometry tubes, and flow cytometry assays were performed according to the manufacturer's instructions.

Apoptosis Detection by Flow Cytometry

Using the Annexin V-FITC/PI Apoptosis Detection Kit (AP101, Multi Sciences, China), supernatants were collected into flow cytometry tubes. Cells were washed with PBS, digested with trypsin, and neutralized with serum-containing medium. Cells were collected and centrifuged at 400 g for 5 minutes at 4°C. Cells were resuspended in 250 μL 1x Binding Buffer, and 2.5 μL Annexin V-FITC and 5 μL PI were added. After a 5-minute incubation in the dark, cells were resuspended in PBS and analyzed by flow cytometry.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 7.0 (https://www.graphpad.com/scientific-software/prism/). Normality tests were conducted on all data, and one-way ANOVA was used for data with normal distribution. P-value less than 0.05 was considered as a statistically significant difference.

Results

Cigarette Smoke Induces an Increase in Cholesterol Levels in Airway Epithelial Cells

To investigate the effect of cigarette smoke on cholesterol levels in airway epithelial cells, we established a classical mouse model induced by cigarette smoke exposure. Wild-type mice were exposed to air and cigarette smoke for 4, 8, and 12 weeks respectively. Cholesterol levels in the lung tissues were detected using a cholesterol assay kit, and we observed an increasing trend in cholesterol levels in the lung tissues of mice exposed to cigarette smoke (Figure 1A). To further examine this phenomenon in vitro, human bronchial epithelial cells (HBE) were treated with cigarette smoke extract (CSE), and the changes in cholesterol content were measured using a cholesterol assay kit. The results showed that CSE induced an increase in total cholesterol content in HBE cells (Figure 1B).

Cigarette Smoke Downregulates Cholesterol Metabolism-Related Molecules in Airway Epithelial Cells

To further explore how cigarette smoke affects cholesterol metabolism in the lungs, we used immunohistochemistry to detect the expression of SREBP2 in mouse lung tissues. We found a reduction in SREBP2 expression in the airway epithelial cells of classical cigarette smoke-induced mice (Figure 2A). SREBP2 is a key regulatory factor in cellular cholesterol biosynthesis. In vitro, HBE cells were treated with CSE, and changes in SREBP2, n-SREBP2, and HMGCR were detected using qPCR and Western blot. n-SREBP2 is the active form of SREBP2, which is cleaved from SREBP2 in the Golgi apparatus and then enters the nucleus to activate the transcription of target genes. We found that CSE

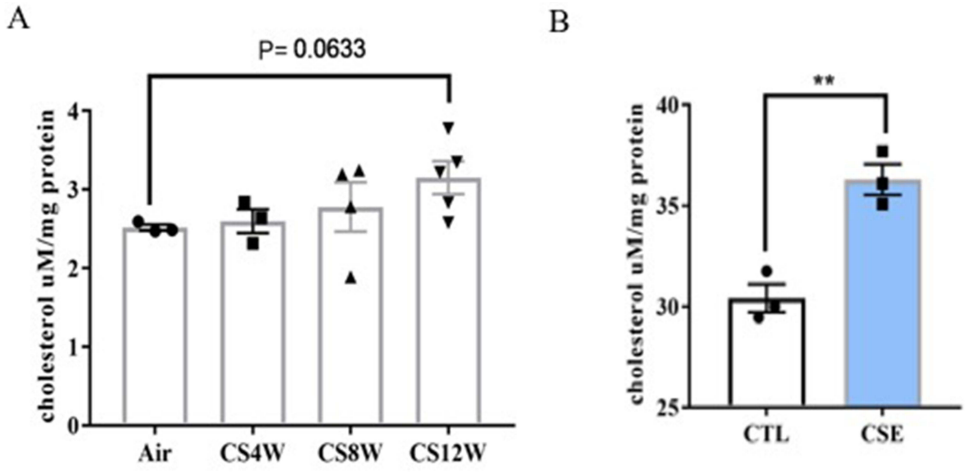

Figure 1 Cholesterol Overload in HBEs and Mice lung tissues After Cigarette Smoke Exposure. Mice were exposed to cigarette smoke (CS) for 4, 8, and 12 weeks, while control mice were exposed to air. Cholesterol content in whole lung tissues (**A**) and in HBEs treated with 1.5% CSE for 6 hours (**B**). Cholesterol levels were normalized to total protein content. Data are presented as mean ± standard error. **P < 0.01.

https://doi.org/10.2147/COPD.S495306

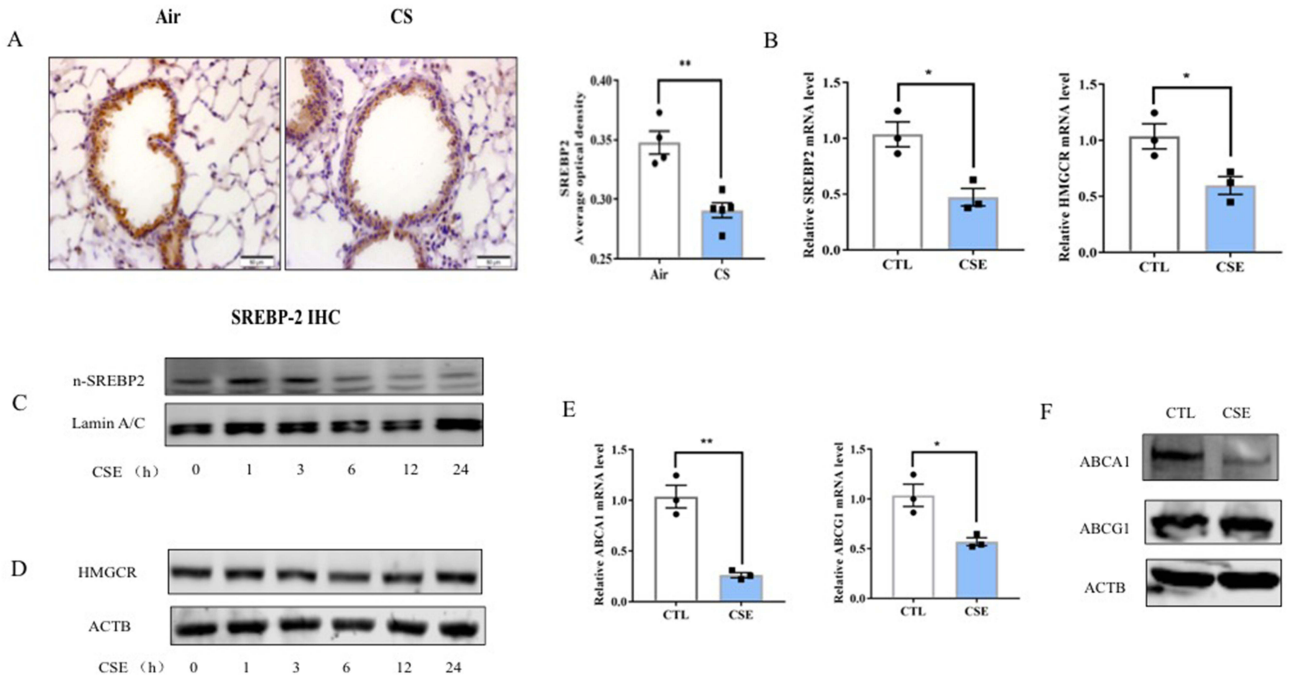

Figure 2 Expression of Cholesterol Metabolism-Related Molecules in Airway Epithelial Cells After Cigarette Smoke Exposure. Mice were exposed to air or cigarette smoke for 3 months. Immunohistochemistry was used to observe changes in SREBP2 in airway epithelial cells of lung tissue (**A**). Representative images were taken at 40x magnification with a diameter of 50 μm (**A**, left). Comparison of average optical density (AOD) of SREBP2 in airway epithelial cells between the two groups (**A**, right). mRNA levels of cholesterol biosynthesis-related molecules SREBP2 (**B**, left) and HMGCR (**B**, right) were measured in HBEs treated with 1.5% CSE for 6 hours. Protein levels of n-SREBP2 (**C**) and HMGCR (**D**) in HBEs treated with 1.5% CSE for 0, 1, 3, 6, 12, and 24 hours. mRNA levels (**E**) and protein levels (**F**) of cholesterol reverse transport-related molecules ABCA1 (**E**, left) and ABCG1 (**E**, right) in HBEs treated with 1.5% CSE for 6 hours. Data are presented as mean ± standard error. *P < 0.05, **P < 0.01.

induced a decrease in the mRNA expression levels of SREBP2 and HMGCR (Figure 2B) and the protein expression level of n-SREBP2 (Figure 2C) in HBE cells, consistent with our in vivo observations (Figure 2A). In addition, the protein level of HMGCR in HBE cells did not decrease significantly (Figure 2D).

However, the total cholesterol content in cells increased, suggesting that other cholesterol metabolism pathways in airway epithelial cells might be affected by cigarette smoke. Therefore, we used qPCR and Western blot to detect the expression of cholesterol efflux transporters ABCA1 and ABCG1. We found that CSE induced a decrease in the mRNA expression levels of ABCA1 and ABCG1 (Figure 2E) and the protein expression level of ABCA1 (Figure 2F) in HBE cells. These results indicate that cigarette smoke extract inhibits both cholesterol efflux and synthesis in airway epithelial cells, ultimately leading to an increase in intracellular cholesterol content.

Reducing Cholesterol Synthesis or Promoting Cholesterol Efflux in Airway Epithelial Cells Protects Against Cigarette Smoke Extract-Induced Inflammation

To further investigate whether changes in intracellular cholesterol levels contribute to the development of cigarette smoke-induced airway inflammation, we silenced the expression of cholesterol biosynthesis-related molecules SREBP2 and HMGCR in HBE cells using siRNA (Figure 3A and B) and inhibited cholesterol biosynthesis in HBE cells using the inhibitor lovastatin (Figure 3F and G). The changes in CSE-induced secretion of inflammatory factors IL6 and IL8 were detected using qPCR, Western blot, and ELISA. Lovastatin targets HMGCR and inhibits cholesterol biosynthesis, commonly used to treat atherosclerosis. We found that inhibiting the expression or function of SREBP2 and HMGCR reduced intracellular cholesterol levels (Figure 3C–F), thereby inhibiting CSE-induced secretion of inflammatory factors IL6 and IL8 in airway epithelial cells (Figure 3D, E and G).

According to the results shown in Figure 3, reducing intracellular cholesterol levels can inhibit CSE-induced secretion of inflammatory factors in HBE cells. To further verify this hypothesis, we used cholesterol-depleting agent methyl-beta-cyclodextrin (MCD) and agonist GW3965 to reduce excess intracellular cholesterol, and detected CSE-induced secretion

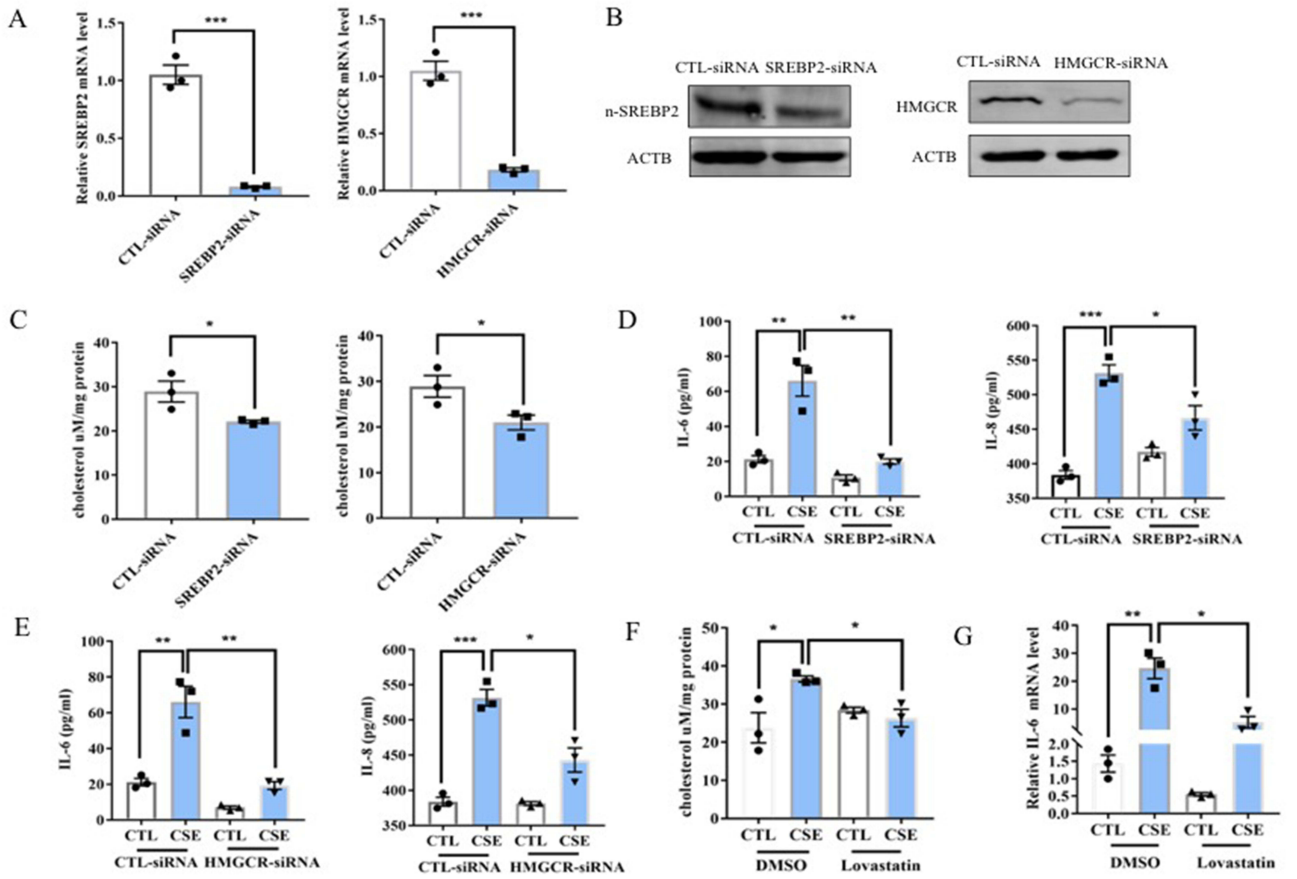

Figure 3 The Effect of Decreased Intracellular Cholesterol Synthesis on Airway Epithelial Inflammation. mRNA levels (**A**) and protein levels (**B**) of SREBP2 and HMGCR in HBEs after knockdown of SREBP2 or HMGCR or negative control for 24 or 48 hours. Intracellular cholesterol content in HBEs after knockdown of SREBP2 or HMGCR or negative control for 24 hours (**C**). Protein levels of IL-6 and IL-8 in HBEs stimulated with 1.5% CSE for 12 hours after knockdown of SREBP2 (**D**) or knockdown of HMGCR or negative control for 24 hours (**E**). Intracellular cholesterol content (**F**) and mRNA levels of IL-6 (**G**) in HBEs after 30 minutes of pretreatment with lovastatin and 12 hours after stimulation with 1.5% CSE. Data are presented as mean ± standard error. *P<0.05, **P<0.01, ***P<0.001.

of inflammatory factor IL6 in HBE cells using qPCR. MCD removes free cholesterol from cells, reducing excess intracellular cholesterol (Figure 4A). GW3965 is a non-selective LXR agonist that upregulates the transcription of ABCA1 and promotes cholesterol efflux (Figure 4C and D). Both agents were able to reduce excess intracellular cholesterol and inhibit CSE-induced secretion of inflammatory factors in HBE cells (Figure 4B and E). Additionally, we found that cigarette smoke exposure induced a decrease in LXRa mRNA levels. Liver X receptor alpha (LXRa) forms a heterodimer with RXR to upregulate ABCA1 expression, and overexpression of LXRa can inhibit CSE-induced secretion of inflammatory factors in HBE cells (Figure 4F and G).

Inhibiting Cholesterol Efflux in Airway Epithelial Cells Exacerbates Cigarette Smoke Extract-Induced Inflammation and Apoptosis

To further verify the role of cholesterol efflux in cigarette smoke-induced airway inflammation, we silenced the expression of cholesterol efflux-related molecule ABCA1 in HBE cells using ABCA1-siRNA (Figure 5A) and detected changes in CSE-induced secretion of inflammatory factors IL6 and IL8 in airway epithelial cells using qPCR, Western blot, and ELISA. We found that inhibiting ABCA1 expression increased intracellular cholesterol levels (Figure 5B), thereby promoting CSE-induced secretion of inflammatory factors IL6 (Figure 5C) and apoptosis (Figure 5D) in airway epithelial cells.

https://doi.org/10.2147/COPD.S495306

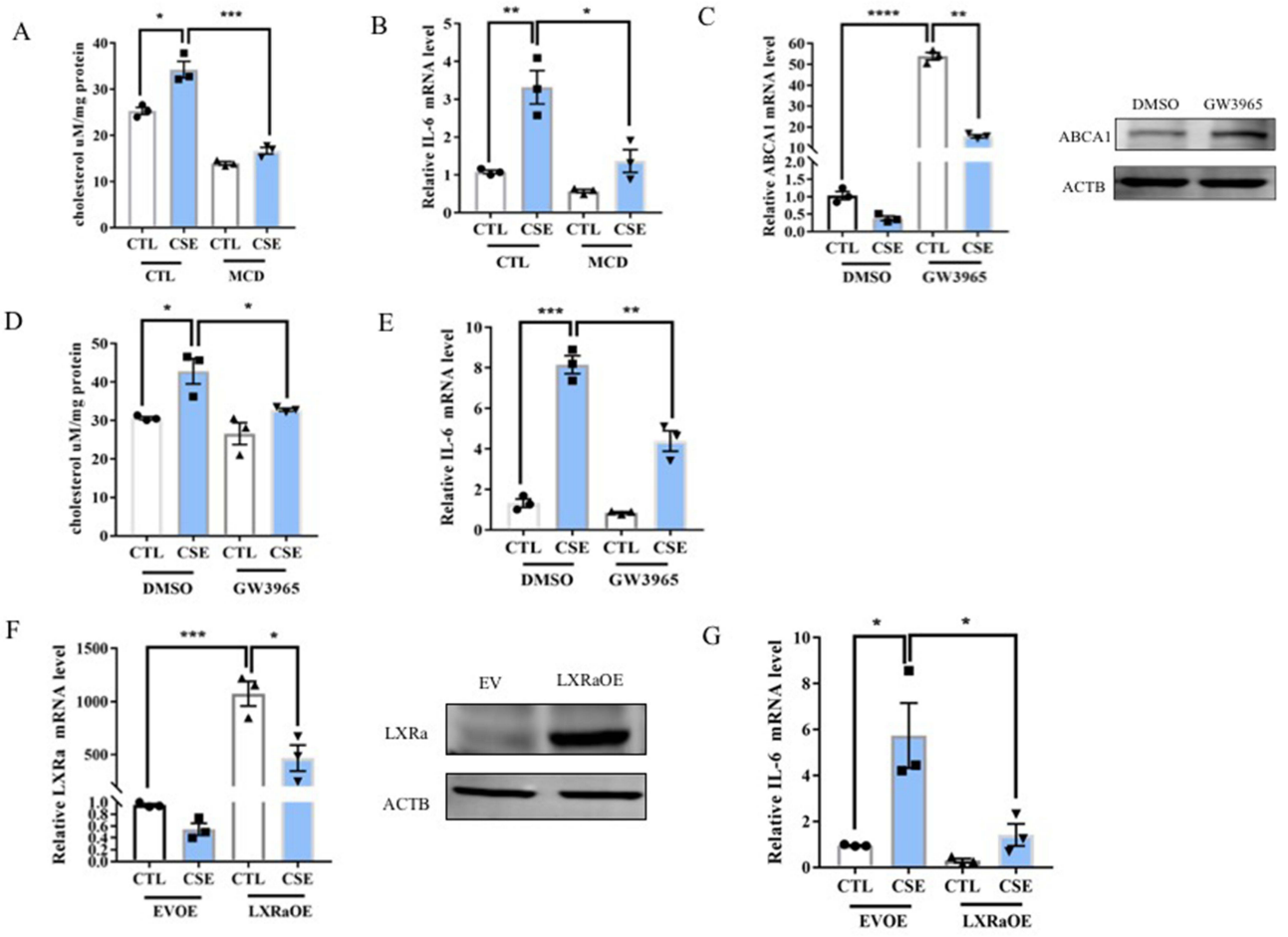

Figure 4 The Effect of Promoting Intracellular Cholesterol Efflux on Airway Epithelial Inflammation. Intracellular cholesterol content (**A**) and mRNA levels of IL-6 (**B**) in HBEs after 2 hours of pretreatment with methyl-β-cyclodextrin (MCD) and 12 hours after stimulation with 1.5% CSE. mRNA and protein expression levels of ABCA1 in HBE after 30 minutes of pretreatment with GW3965 and 12 hours after stimulation with 1.5% CSE (**C**). Intracellular cholesterol content (**D**) and mRNA levels of IL-6 (**E**) in HBEs after 30 minutes of pretreatment with GW3965 and 12 hours after stimulation with 1.5% CSE. mRNA and protein expression levels of LXRα (**F**) and mRNA levels of IL-6 (**G**) in HBEs stimulated with 1.5% CSE for 12 hours after 24 hours of transfection with LXRα-overexpressing plasmid. Data are presented as mean ± standard error. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Accumulated Cholesterol May Regulate Cigarette Smoke-Induced Airway Inflammation by Promoting Oxidative Stress

Studies have shown that cigarette smoke exposure can promote cellular reactive oxygen species (ROS) production and DNA damage, leading to cellular injury.[15] To further verify whether the increase in cholesterol levels induced by cigarette smoke in airway epithelial cells contributes to cigarette smoke-induced airway inflammation through ROS promotion, we treated HBE cells with water-soluble cholesterol and detected ROS levels in airway epithelial cells using flow cytometry (Figure 6A) and the secretion levels of inflammatory factors IL6 and IL8 using qPCR (Figure 6B). The results showed that treatment with water-soluble cholesterol increased ROS and secretion of inflammatory factors in HBE cells.

Our study indicates that cigarette smoke exposure inhibits cholesterol efflux in airway epithelial cells, leading to increased intracellular cholesterol levels, promoting ROS production, and the secretion of inflammatory factors, thereby exacerbating cigarette smoke-induced airway inflammation. Cigarette smoke exposure also inhibited cholesterol synthesis in airway epithelial cells, but ultimately intracellular cholesterol levels increased, suggesting that cigarette smoke inhibited cholesterol efflux more than cholesterol synthesis. Reducing excess intracellular cholesterol by inhibiting cholesterol synthesis and promoting efflux can alleviate CSE-induced secretion of inflammatory factors in airway epithelial cells (Figure 7).

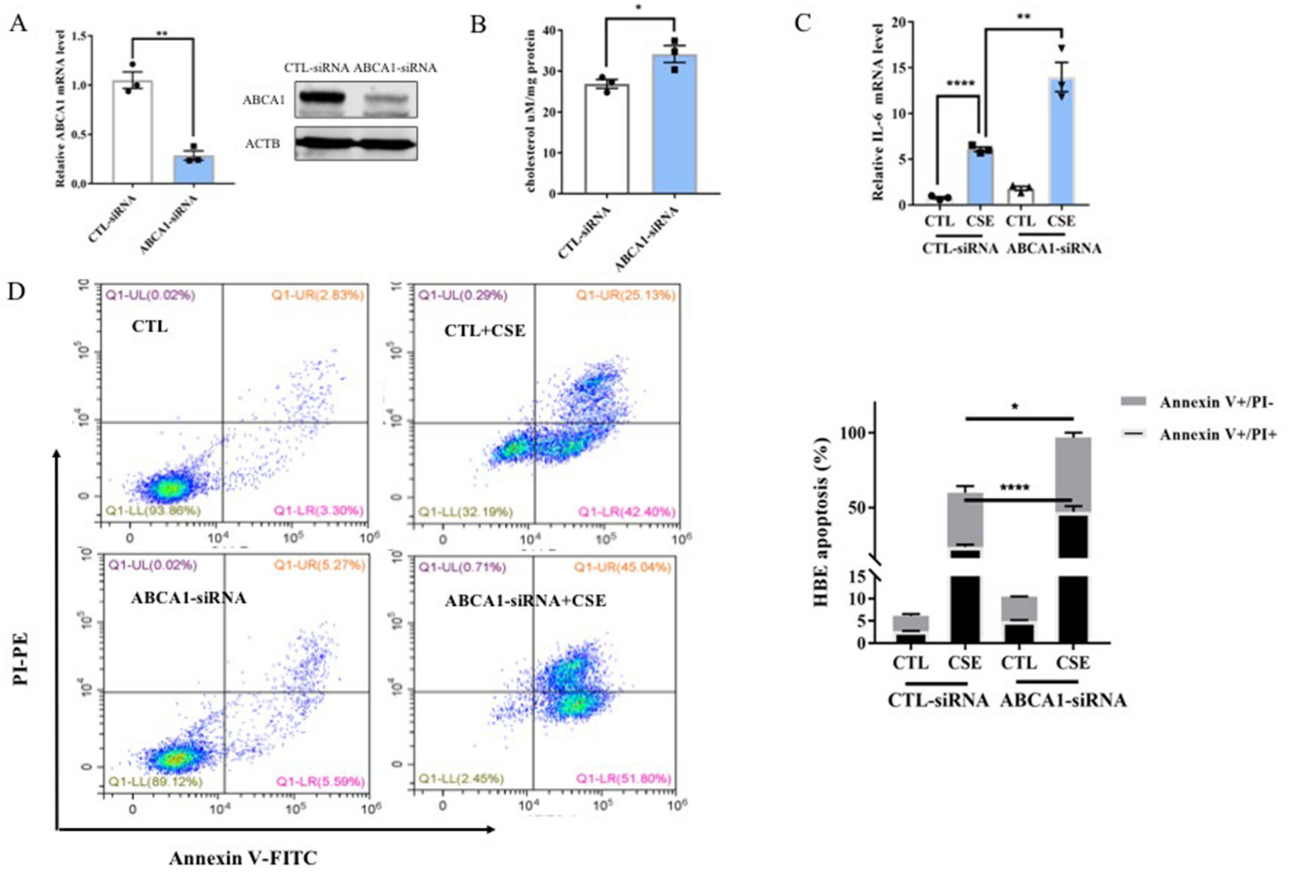

Figure 5 The Effect of Inhibiting Intracellular Cholesterol Efflux on Airway Epithelial Inflammation and Apoptosis. mRNA levels and protein levels of ABCA1 in HBEs after knockdown of ABCA1 or negative control for 24 or 48 hours (**A**). Intracellular cholesterol content in HBEs after knockdown of ABCA1 or negative control for 24 hours (**B**). mRNA levels of IL-6 in HBEs stimulated with 1.5% CSE for 12 hours after knockdown of ABCA1 or negative control for 24 hours (**C**). Apoptotic levels in HBEs stimulated with 5% CSE for 24 hours after knockdown of ABCA1 or negative control for 24 hours (**D**). Data are presented as mean ± standard error. *P<0.05, **P<0.01, ****P<0.0001.

Discussion

In this study, we found that cigarette smoke exposure downregulates ABCA1 expression, inhibiting cholesterol efflux, thereby leading to an increase in cholesterol content within airway epithelial cells. This promotes the generation of reactive oxygen species and the secretion of inflammatory cytokines. A similar phenotype has been reported in macrophages, where cigarette smoke can adversely affect cholesterol transport through an ABCA1-dependent mechanism. This enhances TLR4/Myd88 signaling, upregulates downstream matrix metalloproteinases (MMPs), and exacerbates inflammation in COPD.[16] Additionally, when intracellular cholesterol is excessive, sterol regulatory element-binding protein cleavage-activating protein (SCAP) undergoes conformational changes, preventing SREBP2 from being cleaved in the Golgi apparatus and entering the nucleus to activate target gene transcription. This induces the degradation of HMGCR and squalene monooxygenase, maintaining intracellular cholesterol homeostasis by inhibiting cholesterol biosynthesis.[17]

Although COPD is primarily a lung disease, increasing research has found that patients with COPD exhibit abnormalities in lipid metabolism, which can influence the prognosis of the disease.[18] Cholesterol, a lipid, is crucial not only for forming cell membranes but also as a precursor for bile acids, vitamin D, and various steroid hormones.[9] Therefore, any disruption in cholesterol homeostasis can lead to cellular and tissue damage, contributing to the onset and progression of related diseases.[10] In recent years, it has been discovered that there may be a potential link between cholesterol metabolism disorders and COPD. This finding is significant for further exploring the pathogenesis of COPD and identifying new therapeutic targets for the disease.

https://doi.org/10.2147/COPD.S495306

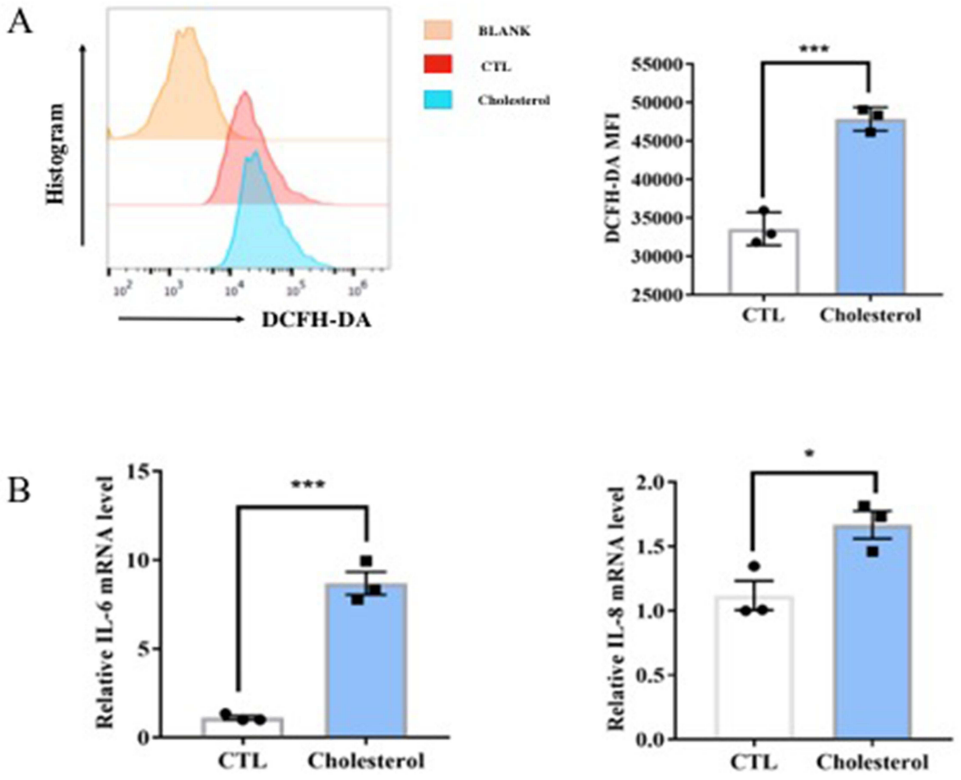

Figure 6 Roles of Cholesterol Overload in Oxidative Stress and Airway Epithelial Inflammation. Intracellular ROS level in HBEs stimulated with extracellular cholesterol for 6 hours (**A**). mRNA levels of IL-6 and IL-8 in HBEs stimulated with extracellular cholesterol for 6 hours (**B**). Data are presented as mean ± standard error. *P<0.001, ***P<0.001.

Exposure to cigarette smoke activates multiple intracellular signaling pathways, leading to cellular damage and disruption of lipid metabolism.[19] Concurrently, changes in lipids and related molecules can in turn regulate specific cellular functions such as oxidative stress and the promotion of inflammatory cytokine secretion, ultimately contributing to the pathogenesis of COPD. In cells and brain tissues lacking NPC1, cholesterol accumulation increases intracellular oxidative stress, which subsequently leads to alterations in mitochondrial function and energy metabolism.[20] Research has reported that Antrodia camphorata can inhibit CSE-induced reactive oxygen species (ROS), exhibiting antioxidative, anti-inflammatory, and anti-atherosclerotic effects.[21] ApoA-I-deficient mice exhibit increased airway resistance, inflammatory cell recruitment, and airway collagen deposition.[22] The absence of ABCA1 or ABCG1 in alveolar type II epithelial cells leads to cholesterol overload, impairing surfactant function, and resulting in severe surfactant protein and lipid accumulation.[23] Liver X receptor (LXR) agonists can alleviate atherosclerosis in animal models by promoting cholesterol efflux and exerting anti-inflammatory effects.[24] It has also been reported that treatment with LXR agonists can restore ABCA1 expression and reduce inflammatory responses following short-term cigarette smoke exposure, and in vitro treatment with LXR agonists can reduce macrophage inflammatory activation induced by cigarette smoke exposure.[16] Statins, which lower cholesterol levels by inhibiting the function of HMGCR, are primarily used to treat atherosclerosis and have been found to possess anti-inflammatory properties in respiratory diseases,[7,8] reducing the incidence and mortality of COPD.[25] Recent studies have found that the cholesterol derivative 25-OH cholesterol can inhibit inflammasome activity, potentially by inhibiting cholesterol or other sterol synthesis, providing new insights into the close link between sterol metabolism and immunity.[26] Additionally, it has been found that external cholesterol may alter the sensitivity to CSE-induced airway epithelial cell inflammation by regulating the STARD3-MFN2 pathway, cholesterol redistribution, changing cholesterol transport and accumulation, the activity of lipid transport regulators, and mitochondrial function and dynamics.[13]

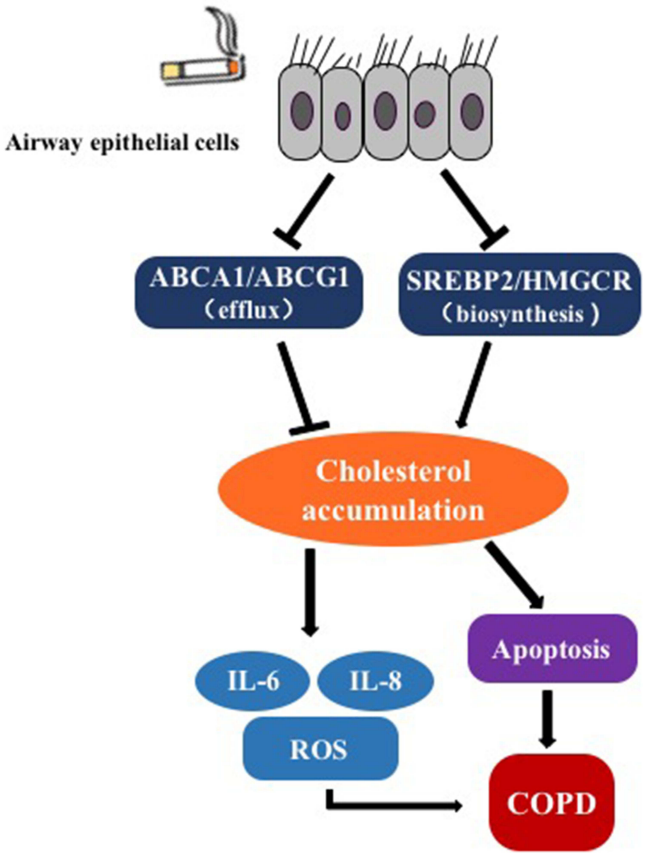

Figure 7 Schematic illustration of cigarette smoke inducing cholesterol accumulation and causing inflammation within airway epithelial cells.
Abbreviations: SREBP2, Sterol Regulatory Element-Binding Protein 2; HMGCR, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; ABCA1, ATP Binding Cassette Transporter A1; ABCG1, ATP Binding Cassette Transporter G1; IL6, Interleukin 6; IL8, Interleukin 8; ROS, reactive oxygen species; COPD, chronic obstructive pulmonary disease.

This study, while providing significant insights into the role of cholesterol metabolism in the pathogenesis of cigarette smoke-induced airway inflammation, is not without its limitations. Firstly, the complexity of cholesterol metabolism and its regulation in epithelial cells in response to cigarette smoke exposure requires more extensive investigation. Although we have identified key signaling pathways and metabolic disruptions, the precise molecular mechanisms and their interactions remain incompletely understood. Additionally, in vitro, we demonstrated that promoting cholesterol efflux, eliminating excess intracellular cholesterol, and inhibiting cholesterol synthesis could suppress cigarette smoke-induced secretion of inflammatory cytokines in airway epithelial cells. However, we have yet to validate these findings in an in vivo model and fully elucidate the mechanisms by which cigarette smoke downregulates ABCA1 expression in airway epithelial cells. Moreover, our results suggest that LXR agonists can reduce cigarette smoke-induced secretion of inflammatory cytokines in airway epithelial cells. Previous literature also reported that treatment with LXR agonists restored ABCA1 expression and mitigated pulmonary inflammation in mice following short-term cigarette smoke exposure. Although LXR agonists have been developed as anti-atherosclerotic drugs, their clinical application is currently under evaluation due to side effects such as fatty liver disease.[27] Thus, there is a need to develop new LXR agonists with limited hepatic targeting. Lastly, statins could be applied to cigarette smoke-induced COPD models to assess COPD-related inflammatory markers. Retrospective studies have suggested that statins may help reduce the incidence and mortality of COPD.[25] However, there is no conclusive evidence that statins provide clinical benefits to COPD patients without cardiovascular risk factors.[28] If our in vivo model validation aligns with the in vitro results, it could indicate the therapeutic potential of statins for the airway inflammation caused by cigarette smoke. Future research should address these limitations by employing advanced techniques, diverse models, and integrative approaches to unravel the complex interplay between cholesterol metabolism and cigarette smoke-induced airway inflammation. For instance, exploring the specific mechanisms by which cigarette smoke regulates the reduction of ABCA1 in

https://doi.org/10.2147/COPD.S495306

airway epithelial cells, and validating these findings in animal or human studies, or investigating the role of elevated intracellular cholesterol levels in promoting reactive oxygen species and cigarette smoke-induced airway inflammation. This will provide new insights into the pathogenesis of COPD and identify new therapeutic targets for clinical treatment.

Funding

This work was supported by grants from the Projects (8180010242 to J.S.Z., 81930003 to H.H.S. and 82100042 to Z.Y.L.) from the National Natural Science Foundation of China.

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Global strategy for the diagnosis, management and prevention of chronic obstructive lung disease, 2024 update.
2. Zhou M, Wang H, Zhu J, et al. Cause-specific mortality for 240 causes in China during 1990–2013: a systematic subnational analysis for the Global Burden of Disease Study 2013. *Lancet*. 2016;387(10015):251–272. doi:10.1016/S0140-6736(15)00551-6
3. Dransfield M, Stolz D, Kleinert S, et al. Towards eradication of chronic obstructive pulmonary disease: a Lancet Commission. *Lancet*. 2019;393 (10183):1786–1788. doi:10.1016/S0140-6736(19)30950-X
4. Proud D, Leigh R. Epithelial cells and airway diseases. *Immunol Rev*. 2011;242(1):186–204. doi:10.1111/j.1600-065X.2011.01033.x
5. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. *Lancet*. 2011;378 (9795):1015–1026. doi:10.1016/S0140-6736(11)60988-4
6. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*. 2004;43 (10):1731–1737. doi:10.1016/j.jacc.2003.12.047
7. Hothersall E, McSharry C, Thomson NC, et al. Potential therapeutic role for statins in respiratory disease. *Thorax*. 2006;61(8):729–734. doi:10.1136/thx.2005.057976
8. Alabed M, Elemam NM, Ramakrishnan RK, et al. Therapeutic effect of statins on airway remodeling during asthma. *Expert Rev Respir Med*. 2022;16(1):17–24. doi:10.1080/17476348.2021.1987890
9. Aguilar-Ballester M, Herrero-Cervera A, Vinué Á, et al. Impact of cholesterol metabolism in immune cell function and atherosclerosis. *Nutrients*. 2020;12(7):2021. doi:10.3390/nu12071986
10. Schade DS, Shey L, Eaton RP. Cholesterol review: a metabolically important molecule. *Endocr Pract*. 2020;26(12):1514–1523. doi:10.4158/EP-2020-0347
11. Luo J, Yang H, Song BL, et al. Mechanisms and regulation of cholesterol homeostasis. *Nat Rev mol Cell Biol*. 2020;21(4):225–245. doi:10.1038/s41580-019-0190-7
12. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nat Rev Immunol*. 2015;15(2):104–116. doi:10.1038/nri3793
13. Li L, Liu Y, Liu X, et al. Regulatory roles of external cholesterol in human airway epithelial mitochondrial function through STARD3 signalling. *Clin Transl Med*. 2022;12(6):e902. doi:10.1002/ctm2.902
14. Zhou JS, Zhao Y, Zhou HB, et al. Autophagy plays an essential role in cigarette smoke-induced expression of MUC5AC in airway epithelium. *Am J Physiol Lung Cell mol Physiol*. 2016;310(11):L1042–52. doi:10.1152/ajplung.00418.2015
15. Scharf P, da Rocha GHO, Sandri S, et al. Immunotoxic mechanisms of cigarette smoke and heat-not-burn tobacco vapor on Jurkat T cell functions. *Environ Pollut*. 2021;268(Pt B):115863. doi:10.1016/j.envpol.2020.115863
16. Sonett J, Goldklang M, Sklepkiewicz P, et al. A critical role for ABC transporters in persistent lung inflammation in the development of emphysema after smoke exposure. *FASEB J*. 2018;32:fj201701381. doi:10.1096/fj.201701381
17. Repa JJ, Mangelsdorf DJ. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. *Annu Rev Cell Dev Biol*. 2000;16:459–481. doi:10.1146/annurev.cellbio.16.1.459
18. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J*. 2009;33(5):1165–1185. doi:10.1183/09031936.00128008
19. Tweed JO, Hsia SH, Lutfy K, et al. The endocrine effects of nicotine and cigarette smoke. *Trends Endocrinol Metab*. 2012;23(7):334–342. doi:10.1016/j.tem.2012.03.006
20. Kennedy BE, Madreiter CT, Vishnu N, et al. Adaptations of energy metabolism associated with increased levels of mitochondrial cholesterol in Niemann-Pick type C1-deficient cells. *J Biol Chem*. 2014;289(23):16278–16289. doi:10.1074/jbc.M114.559914
21. Yang HL, Korivi M, Chen CH, et al. Antrodia camphorata attenuates cigarette smoke-induced ROS production, DNA damage, apoptosis, and inflammation in vascular smooth muscle cells, and atherosclerosis in ApoE-deficient mice. *Environ Toxicol*. 2017;32(8):2070–2084. doi:10.1002/tox.22422
22. Wang W, Xu H, Shi Y, et al. Genetic deletion of apolipoprotein A-I increases airway hyperresponsiveness, inflammation, and collagen deposition in the lung. *J Lipid Res*. 2010;51:2560–2570. doi:10.1194/jlr.M004549
23. Gowdy KM, Fessler MB. Emerging roles for cholesterol and lipoproteins in lung disease. *Pulm Pharmacol Ther*. 2013;26(4):430–437. doi:10.1016/j.pupt.2012.06.002
24. Claudel T, Leibowitz MD, Fiévet C, et al. Reduction of atherosclerosis in apolipoprotein E knockout mice by activation of the retinoid X receptor. *Proc Natl Acad Sci USA*. 2001;98(5):2610–2615. doi:10.1073/pnas.041609298
25. Mancini GB, Etminan M, Zhang B, et al. Reduction of morbidity and mortality by statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers in patients with chronic obstructive pulmonary disease. *J Am Coll Cardiol*. 2006;47(12):2554–2560. doi:10.1016/j.jacc.2006.04.039

26. Reboldi A, Dang EV, McDonald JG, et al. Inflammation. 25-Hydroxycholesterol suppresses interleukin-1-driven inflammation downstream of type I interferon. *Science*. 2014;345(6197):679–684. doi:10.1126/science.1254790
27. Hong C, Tontonoz P. Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nat Rev Drug Discov*. 2014;13(6):433–444. doi:10.1038/nrd4280
28. Howard ML, Vincent AH. Statin effects on exacerbation rates, mortality, and inflammatory markers in patients with chronic obstructive pulmonary disease: a review of prospective studies. *Pharmacotherapy*. 2016;36(5):536–547. doi:10.1002/phar.1740