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ORIGINAL RESEARCH

TET1 Alleviates Cigarette Smoke Induced Bronchial Epithelial Cell Apoptosis Through Upregulating Nrf2

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Introduction: Nuclear factor erythroid 2–related factor 2 (Nrf2), a key regulator of oxidative stress responses, is downregulated in patients with GOLD stage III–IV chronic obstructive pulmonary disease (COPD). However, the mechanisms underlying the epigenetic regulation of Nrf2 in COPD remain poorly understood.

Methods: Protein levels of Nrf2, heme oxygenase-1 (HO-1), ten–eleven translocation methylcytosine dioxygenase 1 (TET1), and DNA methyltransferase 1 (DNMT1) were assessed by Western blotting in peripheral lung tissue and primary bronchial epithelial cells obtained from patients with COPD, never-smokers (control-NS), and smokers without COPD (control-S). CSE-treated human bronchial epithelial (HBE) cells were used as an in vitro model. Nrf2 promoter methylation was evaluated using bisulfite sequencing. Apoptosis of HBE cells was measured by flow cytometry. Chromatin immunoprecipitation (ChIP) was performed to assess the binding of TET1 to the Nrf2 promoter. Malondialdehyde (MDA) and superoxide dismutase (SOD) activity assays were used to quantify oxidative stress and antioxidant capacity.

Results: Nrf2 and HO-1 expression was significantly reduced in both lung tissue and primary epithelial cells from patients with COPD. In vitro, CSE exposure increased Nrf2 promoter methylation in HBE cells. Overexpression of Nrf2 mitigated oxidative stress, increased SOD activity, and reduced apoptosis in response to CSE. TET1 expression was decreased in COPD lungs, and TET1 was shown to bind the Nrf2 promoter and enhance its transcription. TET1 overexpression reduced oxidative damage and apoptosis via Nrf2 upregulation.

Conclusion: Reduced Nrf2 expression in COPD may result from promoter hypermethylation. TET1 directly binds and demethylates the Nrf2 promoter, restoring its expression and attenuating CSE-induced HBE cells apoptosis. These findings identify a potential epigenetic mechanism contributing to COPD pathogenesis and suggest TET1 as a novel therapeutic target.

Keywords: chronic obstructive pulmonary disease, Nrf2, TET1, DNA methylation

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory airway disease with high morbidity and mortality. Oxidative stress has been recognized as an important predisposing factor that accounts for the pathogenesis of COPD. Long-term inhalation of cigarette smoke and other noxious gases is the main causal mechanism underlying irreversible destruction of the respiratory system, which is associated with oxidative stress.[1–3] Bronchial epithelial cells are the cells to encounter inhaled pathogens and environmental pollutants or irritants which serve as the innate immune system in COPD development. Cigarette smoke-induced oxidative stress has been shown to trigger apoptosis of bronchial epithelial cells, contributing to lung parenchyma destruction and emphysema development.[4,5] Among anti-oxidant defense pathways, nuclear factor erythroid 2–related factor 2 (Nrf2) plays a critical protective role in the pathophysiology of COPD.[6]

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Nrf2 is a major transcription factor that counteracts oxidative stress and inflammation. Nrf2 is normally bound to its inhibitor, Kelch-like ECH-associated protein-1 (KEAP1). Under oxidant conditions, Nrf2 disassociates with KEAP1 and induces antioxidant response element (ARE) driven cytoprotective gene transcription, including glutathione peroxidase (GPX), heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase (NQO1).[7] In patients with emphysema, Nrf2 expression is significantly reduced in lung tissue and alveolar macrophages,[8] whereas in healthy smokers, the Nrf2 pathway is typically activated to counterbalance oxidative burden.[7] Experimental activation of Nrf2 via Keap1 deletion in the airway epithelium significantly increased pulmonary glutathione levels and attenuated cigarette smoke–induced oxidative stress and inflammation in vivo.[9] The potential role of Nrf2 in modulating oxidative stress warrants further investigation to inform therapeutic strategies for COPD.

Apoptosis a tightly regulated mechanism of cell death. Several studies suggest that apoptosis of structural lung cells, including epithelial and endothelial cells, may play a role the pathogenesis of COPD.[4] Apoptosis is mediated by several pathways. The Bax/Bcl-2 pathway is involved in oxidative stress and mitochondrial apoptosis.[10] Elevated ROS disrupt the Bax/Bcl-2 ratio, promoting mitochondrial outer membrane permeabilization, cytochrome c release, and caspase activation, ultimately leading to programmed cell death.[11,12] The molecular mechanisms underlying bronchial epithelial cell apoptosis in COPD merit further investigation.

DNA methylation at the C5 position of cytosine (5methylcytosine, 5mC) is a crucial epigenetic modification that has been implicated in numerous cellular processes in mammals, including transcription, and abnormal methylation changes are involved in a wide spectrum of malignant and nonmalignant diseases.[13] The patterns of DNA methylation in cells are initially established by DNA methyltransferases DNMT3a and DNMT3b, and then faithfully maintained during DNA replication by the maintenance methyltransferase DNMT1. The ten-eleven translocation (TET) family proteins were identified as 5mC dioxygenases which can induce passive or active DNA demethylation in genomic DNA. The dysregulation of the expression of these enzymes is in conjunction with the development of some human disorders, including cancers, neurodegenerative diseases, and developmental pathologies.[14] Disruption of DNA methylation was observed in various diseases in response to cigarette smoke.[15] Accordingly, CpG hypermethylation of the promoter has similarly been found to decrease Nrf2 levels in the lung in COPD.[16,17] However, the upstream regulators responsible for this modification remain unclear.

The aim of this study was to validate the pivotal effect of Nrf2 in regulating oxidative stress in COPD and the molecular mechanisms involved in Nrf2 hypermethylation. In the present study, we investigated the function of TET1 in modulating Nrf2 expression and its downstream effects on oxidative damage and bronchial epithelial cell apoptosis. Our findings on the methylation of the Nrf2 promoter in the pathogenesis of COPD may contribute to the identification of novel therapeutic targets for the disease.

Materials and Methods

Study Participants and Specimens

The subjects from Wuxi People's Hospital were divided into three groups: (1) nonsmokers without COPD (Control-NS), (2) smokers without COPD (Control-S), and (3) COPD patients. This study followed the guidelines of the Global Initiative for Chronic Obstructive Pulmonary Disease for the diagnosis of COPD. Lung tissue samples were obtained from patients who had received lobectomy or lung transplantation at Wuxi People's Hospital. All lung tissue samples were immediately frozen in liquid nitrogen for further detection.

The primary bronchial epithelial cells were obtained from patients who received fiberoptic bronchoscopy for pneumonia or a small amount of bloody sputum at Wuxi People's Hospital. Primary bronchial epithelial cells were obtained by five consecutive brushing over the bronchial mucosa of the second- and third-generation bronchi. After each brushing, the cells were resuspended in RPMI 1640 medium (HyClone, USA) with 1% penicillin/streptomycin (P/S) and then mucus was filtered out using a cell strainer (Falcon, USA). The filtered cells were washed twice with RPMI 1640 medium and then resuspended and cultured in BEGM (Lonza, USA) medium and seeded into a 6-well plate. The cells were cultured in a 37°C incubator with 5% CO_2, and the medium was replaced every 2–3 days. When the cells reached 80–90% confluence, they were passaged. During passaging, 0.05% trypsin was added for digestion. The primary bronchial epithelial cells were passaged until the 2nd or 3rd generation, after which they were gently scraped with a cell scraper and stored at −80°C until further use.

The characteristics of the lung tissues are shown in Table 1, and the characteristics of the bronchial epithelial cells are shown in Table 2.

This study was approved by the Ethics Committee of the Affiliated Wuxi People's Hospital of Nanjing Medical University and was conducted in accordance with the Declaration of Helsinki. Written informed consent was signed for all subjects.

Cell Culture and Treatment

Human bronchial epithelial (HBE) cells were obtained from ATCC (USA) and cultured in DMEM supplemented with a penicillin/streptomycin (P/S) solution. Cigarette smoke extract (CSE) was prepared and diluted to a 5% concentration using DMEM without fetal bovine serum (FBS). HBE cells were exposed to 5% CSE for 72 hours upon reaching 70–80% confluence.

Preparation of CSE

The smoke from a cigarette was bubbled through 10 mL of DMEM without FBS. The resultant solution was regarded as 100% CSE. The absorbance of the CSE solution at A540 was monitored, and the CSE solution was considered acceptable when the A540 was between 0.9 and 1.2. Then, the solution was filtered through a 0.22-μm pore filter and diluted with DMEM medium for use within 30 minutes.

Western Blot Analysis

Proteins collected from human lung tissue, primary bronchial epithelial cells and HBE cells were resolved by 10% SDS–PAGE and transferred to PVDF membranes. The membranes were then incubated with antibodies against Nrf2 (Abcam, United States), HO-1 (Cell Signaling Technology, United States), ten-eleven translocation 1 (TET1) (Sigma–Aldrich, United States), DNA methyltransferase 1 (DNMT1) (Abcam, United States), B-cell lymphoma 2 (Bcl-2) (Cell Signaling Technology, United States), BCL2-Associated X (Bax) (Cell Signaling Technology, United States) and β-actin (Proteintech, China). Band density was detected by ImageJ.

Table 1 Characteristics of Lung Tissues Examined in the Study (Data Presented as the Mean ± SD)

	Nonsmokers without COPD (Control-NS)	Smokers without COPD (Control-S)	Patients with COPD
Number	9	6	7
Male, n (%)	4 (44.4%)	5 (83.3%)	6 (85.7%)
Age (years)	59±13	64±3	60±3
Smoking (pack-years)	0	24±16	22±6
FEV1%pred	95.6±11.3	91.2±5.7	17.9±5.3
FEV1/FVC (%)	89.1±5.1	79.1±2.4	35.2±3.2

Table 2 Characteristics of the Bronchial Epithelial Cells Used in the Study (Data Presented as the Mean ± SD)

	Nonsmokers without COPD (control-NS)	Smokers without COPD (Control-S)	Patients with COPD
Number	15	9	12
Male, n (%)	9 (60%)	9 (100%)	12 (100%)
Age (years)	50±17	61±11	65±8
Smoking (pack-years)	0	39±21	42±20
FEV1%pred	94.3±12.2	87.9±4.0	42.6±17.7
FEV1/FVC (%)	86.6±2.3	77.6±1.9	43.4±9.7

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Immunohistochemistry (IHC)

The lung tissues were fixed in 10% formalin (Beyotime, China) for 24 h at room temperature. The fixed tissues were then sliced and embedded in paraffin. Glass slides carrying tissue sections (4 μm) were dried for 2 h at 60 °C. Then, the slides were deparaffinized with dimethylbenzene and rehydrated with graded ethanol. Next, heat-induced retrieval was performed with citrate antigen retrieval solution for 10 min at 100 °C. The slides were incubated with anti-TET1 antibody (1:500 dilution, GeneTex, USA) overnight and stained with DAB. Images were captured with an Olympus microscope (Olympus IX71, Japan). Immunostaining results were scored as the integrated optical density (IOD)/ area as detected by Image-Pro Plus.

Nrf2 Activity Assay

Nuclear extracts were obtained with a Nuclear and Cytoplasmic Extraction Kit (Cwbio, China). A TransAM™ Nrf2 kit (Active Motif, USA) was used to detect the binding of Nrf2 to immobilized AREs. Ten microliters of each sample (10 μg) was diluted with 40 μL of complete binding buffer. The plate, which had been precoated with DNA probes, was covered and incubated for 1 hour at 100 rpm. After 3 washes, anti-Nrf2 antibody (1:1000 dilution, Abcam, United States) was added to the nuclear extracts and incubated for 1 hour. After washing 3 times again, HRP-conjugated antibody (1:1000 dilution) was added to all wells and incubated for 1 hour. A450 was read on a spectrophotometer, with a reference wavelength of 655 nm.

Cell Transfection

TET1 mimic and control mimic were transfected into HBE cells using Lipofectamine 3000 (Invitrogen, USA). After 56 hours, HBE cells were incubated with 5% CSE for 72 hours. The cells were harvested for further experiments.

MDA Assay

HBE cells were collected in cell lysis buffer (Beyotime, China). The sonicated supernatant was collected for the MDA determination (Beyotime, China). MDA levels were detected by measuring the absorbance at 532 nm.

Superoxide Dismutase (SOD) Assay

The HBE cell extracts were used to assess the relative SOD concentration via a SOD assay kit (Nanjing Jiancheng Bioengineering Institute, China).

Cell Proliferation Assay

A total of 4×10^3 cells were plated in 96-well plates per well. 10 μL of CCK-8 solution was added to each well and then incubated for 2 h at the time of 24, 48, and 72 h. Proliferation was assessed by measuring the absorbance at a wavelength of 450 nm.

Flow Cytometry

Cell apoptosis was detected with an Annexin V-fluorescein isothiocyanate (APC)/7-AAD kit (Keygen, China). HBE cells were harvested and suspended in 500 μL of binding buffer. The samples were added with 5 μL of Annexin V-APC and 5 μL of 7-AAD separately in the dark, then incubated for 15 min. The cells were immediately analyzed by using flow cytometry (BD FACSCanto, USA). The percentage of apoptotic cells was calculated as the proportion of cells in Q2 (Annexin V-APC, 7-AAD+) + the proportion of cells in Q3 (Annexin V-APC+, 7-AAD-).

ChIP Assay

The sites of the Nrf2 promoter to which TET1 binds were predicted by ChIP-seq (GSM2642522) on the Cistrome Data Browser. The ChIP assay was performed with the SimpleChIP enzymatic chromatin IP kit (Cell Signaling Technology, USA). Cells were crosslinked by 1% formaldehyde for 12 min at room temperature. After sonicated and treated with nuclease, chromatin immunoprecipitation was performed with an antibody against TET1 (GeneTex, USA) and IgG. Chromatin digests were incubated with 7.5 μg of anti-TET1 antibody and IgG overnight at 4 °C. The immunoprecipitates were captured with protein G magnetic beads, washed and then eluted with elution buffer. The immunoprecipitated DNA was purified and subjected to 40 cycles of PCR. The cycling conditions were as follows: 95 °C for 3 min, then 40 cycles

of 15s at 95 °C and final extension for 60s at 60 °C. The following primers specific for the Nrf2 gene promoter were used: forward 5′-AAGGCGTTGGTGTAGGAGC-3′, reverse 5′-CATTCTCGGGCGGTAAAGTG-3′.

Bisulfite Sequencing

Total DNA was extracted and then subjected to bisulfite conversion with an EpiTect® Bisulfite kit (59104, Qiagen). Each sample contained plasmid DNA from at least 10 colonies and was prepared using a BiQ Analyzer before sequencing.

Statistical Analysis

All the data are expressed as the mean ± SD. Student's *t* test was used for two-group comparisons (with a normal distribution). Three or more groups were analyzed using one-way ANOVA accompanied by the Bonferroni post hoc test (equal variances assumed) or Dunnett's T3 (equal variances not assumed) post hoc test. Differences for which P <0.05 were considered to be statistically significant.

Results

Nrf2 Levels in the Lung Were Decreased in COPD

To identify the level of Nrf2 in COPD, Western blotting was used to detect Nrf2 protein expression in human lung tissue. Nrf2 levels were significantly decreased in COPD lung tissue (Figure 1A and B). In addition, we collected primary bronchial epithelial cells from patients scheduled for bronchoscopy. Consistent with the findings in human lung tissue, Nrf2 was downregulated in primary bronchial epithelial cells from COPD patients (Figure 1D and E). Nrf2 translocates to the nucleus, binding the AREs and activates transcription under stress conditions. The level of one target of Nrf2, HO-1, in COPD was decreased in both lung tissue (Figure 1A and C) and primary bronchial epithelial cells (Figure 1D and F). We also examined Nrf2 activity using a Nrf2 activity assay. The level of Nrf2 activity was lower in COPD primary epithelial cells than in normal cells and Control-S subjects (Figure 1G). This suggested that Nrf2 activity was inhibited in patients with severe COPD.

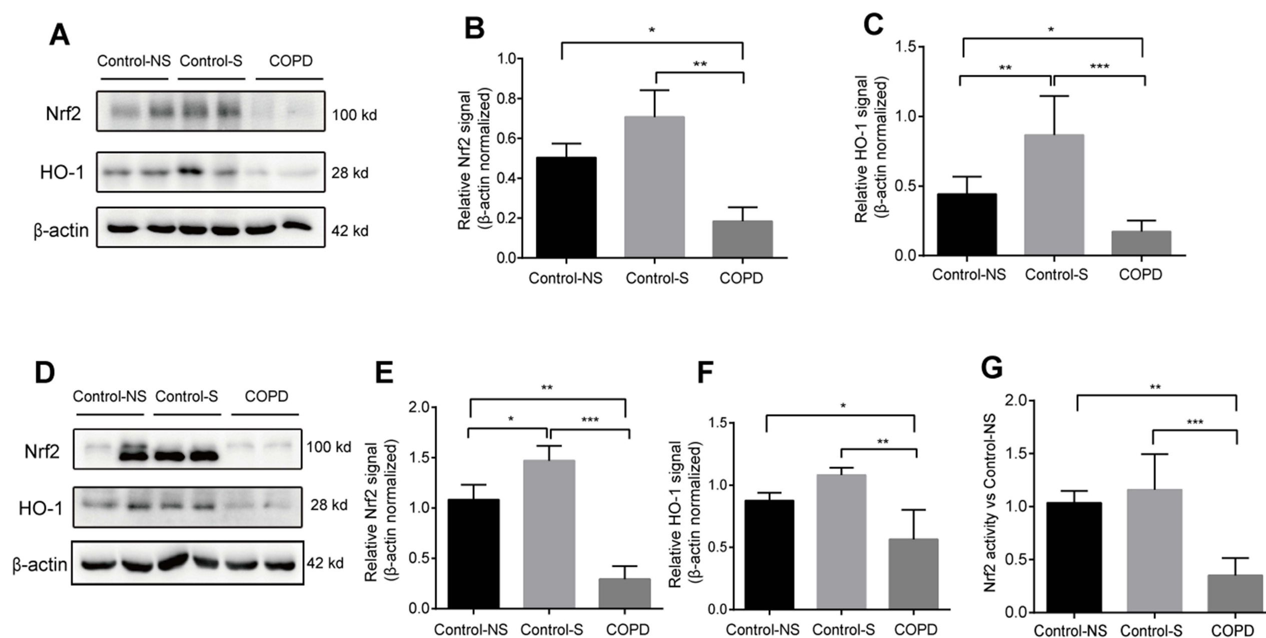

Figure 1 Nrf2 levels in the lung were decreased in COPD. (**A-C**) Western blots of human lung homogenates from healthy volunteers (Control-NS) (n=9), healthy smokers without COPD (Control-S) (n=6) and COPD patients (n=7) were probed using (**B**) anti-Nrf2 and (**C**) anti-HO-1 antibodies; the values were normalized to β-actin levels (loading control). (**D-F**) Western blots of human primary bronchial epithelial cells from the Control-NS (n=15) and Control-S (n=9) groups and COPD patients (n=12) were probed using (**E**) anti-Nrf2 and (**F**) anti-HO-1 antibodies; the values were normalized to β-actin levels (loading control). (**G**) Nrf2 activity of human primary bronchial epithelial cells from the Control-NS and Control-S groups and COPD patients. *P<0.05; **P < 0.01; ***P < 0.001.

Downregulation of Nrf2 Contributed to Apoptosis in CSE-Treated HBE Cells

We found that Nrf2 levels were decreased in COPD human lung tissue and primary bronchial epithelial cells. In vitro, Nrf2 was downregulated in HBE cells exposed to CSE for 72 h, accompanied by the downregulation of HO-1 (Figure 2A–C). CSE increased Bax (Figure 2D and E) and decreased Bcl-2 (Figure 2D and F) expression, accompanied by an elevated proportion of apoptotic cells (Figure 2G and H). Cell viability was also decreased (Figure 2I) in response to CSE. Increased ROS levels, as detected by the MDA assay results (Figure 2J), and impairment of the antioxidant activity of SOD (Figure 2K) were observed. However, sulforaphane (SFN), an Nrf2 agonist, restored the activity of Nrf2 and alleviated the change in apoptosis (Figure 2G and H).

The Nrf2 Promoter Was Hypermethylated in CSE-Treated HBE Cells

DNA methylation occurs at CpG islands and functions to regulate gene transcription and maintain transposon inactivation, which is critical for normal cellular development.[18] Bisulfite sequencing was performed to determine the effect of CSE on the methylation of the Nrf2 promoter in HBE cells. We detected CpG sites in the Nrf2 promoter from −500 to −1 bps. The level of Nrf2 promoter methylation was upregulated upon stimulation with CSE (Figure 3).

DNA Methylation-Related Proteins in CSE-Treated HBE Cells

Based on data from clinical samples, we explored the molecular mechanisms underlying the reduction in Nrf2 levels using HBE cells in vitro. DNA methyltransferases (DNMTs) are associated with DNA methylation, and tet methylcytosine dioxygenase 1(TET) proteins are associated with DNA demethylases. Next, the expression of DNMT1 (Figure 4A and B) and TET1 (Figure 4A and C) was investigated. The effects of incubation with 5% CSE for 24 h, 48 h and 72 h on the levels of these proteins in HBE cells were determined. There was no significant difference in DNMT1 expression between the two groups and did not show a time-dependent relationship during CSE treatment, whereas TET1 expression was reduced in CSE-treated HBE cells compared with control HBE cells.

TET1 Levels Were Decreased in the Lungs of COPD Patients

As shown in Figure 5A and B, the TET1 level was decreased in COPD lung homogenates, as detected by Western blotting. Using IHC staining, we found that TET1 expression was downregulated, and positive staining for TET1 was primarily located in epithelial cells in the lung tissues, suggesting that epithelial cells are an important target and the cell type in which TET1 levels are reduced (Figure 5C and D).

TET1 Upregulated Nrf2 by Binding the Nrf2 Promoter in CSE-Treated HBE Cells

ChIP assays showed that TET1 could bind the Nrf2 promoter, but CSE impaired the interaction between TET1 and Nrf2 (Figure 6A). To confirm the regulatory effect of TET1 on Nrf2, we transfected a TET1 mimic and found that TET1 increased the protein expression of Nrf2 (Figure 6B–D). These results indicated that TET1 upregulated Nrf2, probably by normal demethylation.

TET1 Alleviated Apoptosis by Upregulating Nrf2

To determine the mechanism of TET1 in COPD pathogenesis, we transfected the TET1 mimic and an NC mimic into HBE cells. TET1 overexpression increased Nrf2 and HO-1 levels in 5% CSE-treated HBE cells (Figure 7A–C). Correspondingly, TET1 decreased Bax levels (Figure 7D and E) and increased Bcl-2 levels (Figure 7D and F) and the apoptosis rate (Figure 7G and H) in response to CSE. TET1 alleviated oxidative stress induced by CSE, as MDA levels (Figure 7J) and SOD activity (Figure 7K) were enhanced upon TET1 overexpression, accompanied by an increase in cell viability (Figure 7I). However, an Nrf2 pathway inhibitor, ML385, reversed the effects of TET1, indicating that the protective effects of TET1 were due to the upregulation of Nrf2/ARE.

Discussion

COPD is a chronic airway disease that is highly correlated with oxidative stress. The levels of a key factor in antioxidative stress, Nrf2, were found to be decreased in COPD, and Nrf2 alleviated apoptosis in CSE-treated HBE

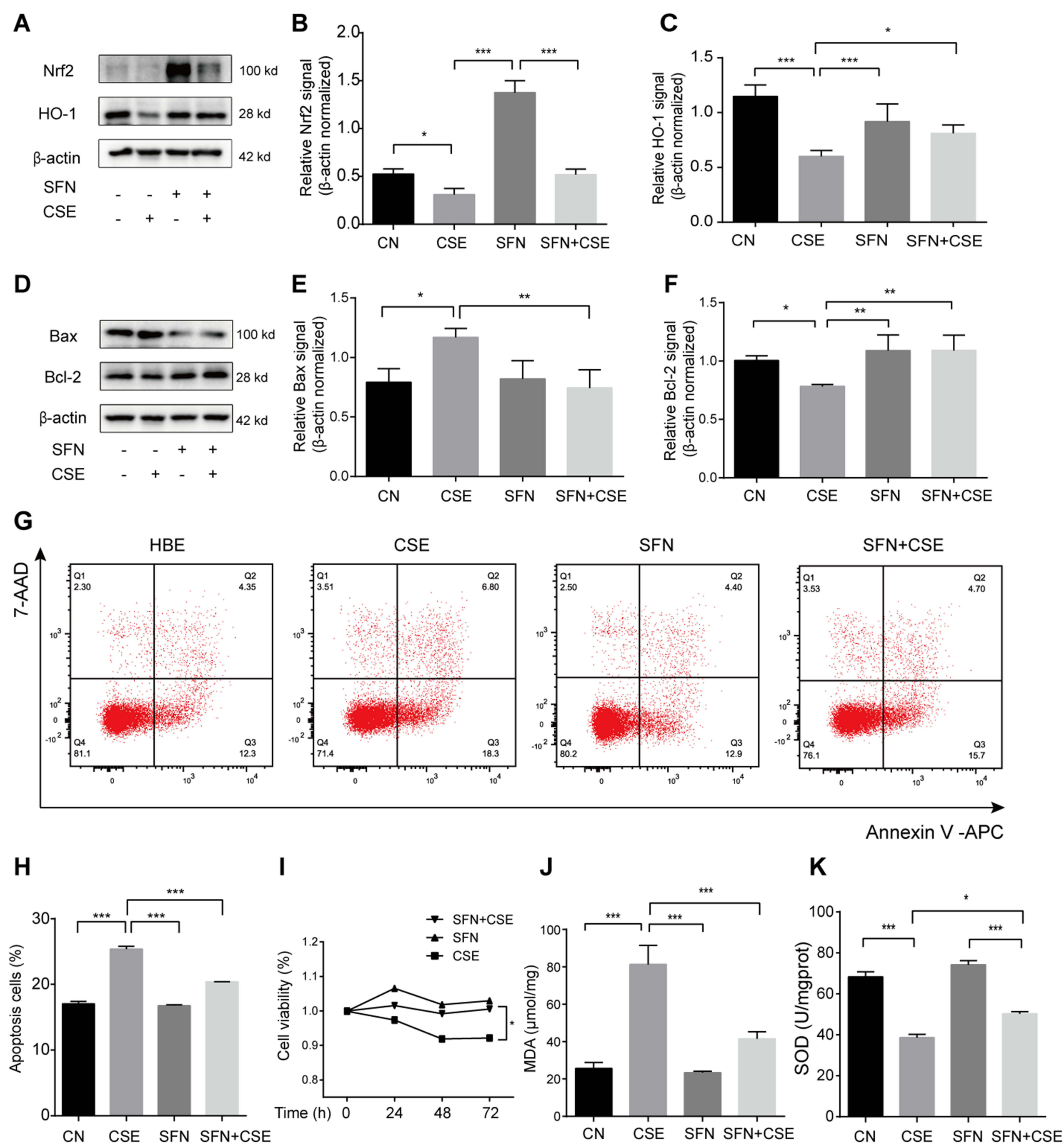

Figure 2 Downregulation of Nrf2 contributed to apoptosis in CSE-treated HBE cells. HBE cells were treated with 5% CSE for 72 h with or without SFN (5 μmol/L). (**A-C**) Western blots of HBE cells were probed using (**A**) anti-Nrf2 or (**C**) anti-HO-1 antibodies; the values were normalized to β-actin levels. (n=3) (**D-F**) Western blots of HBE cells were probed using (**E**) anti-Bax or (**F**) anti-Bcl-2 antibodies; the values were normalized to β-actin levels. (n=3) (**G** and **H**) Apoptotic HBE cells were detected using Annexin V-APC/7-AAD staining and flow cytometry. (n=3) (**I**) The cell viability of each group was analyzed by the CCK-8 assay, and all values were normalized to those of the control group. (n=3) (**J**) MDA levels and (**K**) SOD activity in each group. (n=3) *P<0.05; **P < 0.01; ***P < 0.001.

cells in this research. In addition, we found that Nrf2 was hypermethylated in CSE-treated HBE cells, probably resulting in the low level of Nrf2 observed in COPD. However, TET1, which plays a role in DNA demethylation, could bind the promoter of Nrf2 and enhance the level of Nrf2 in HBE cells, thus preventing apoptosis induced by CSE (Figure 8). This study reveals the role of TET1 in the pathogenesis of COPD.

Figure 3 The Nrf2 promoter was hypermethylated in CSE-treated HBE cells. (**A**) The level of methylation in the promoter of Nrf2 in HBE cells treated with 0% CSE or (**B**) 5% CSE for 72 h was detected with bisulfite sequencing.

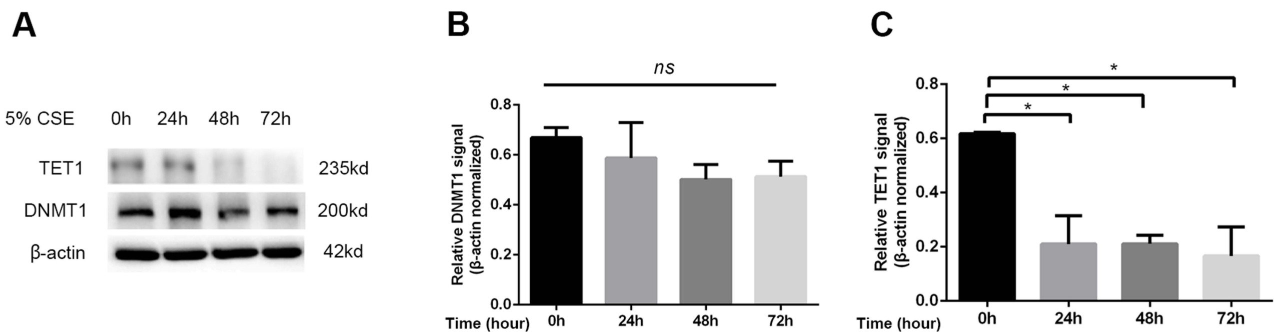

Figure 4 DNA methylation-related proteins in CSE-treated HBE cells. (**A**) HBE cells were stimulated with 5% CSE for 0, 24, 48, and 72 h. Western blots of HBE cells were probed using (**B**) anti-DNMT1 or (**C**) anti-TET1 antibodies; the values were normalized to β-actin levels. (n=3) *P<0.05.

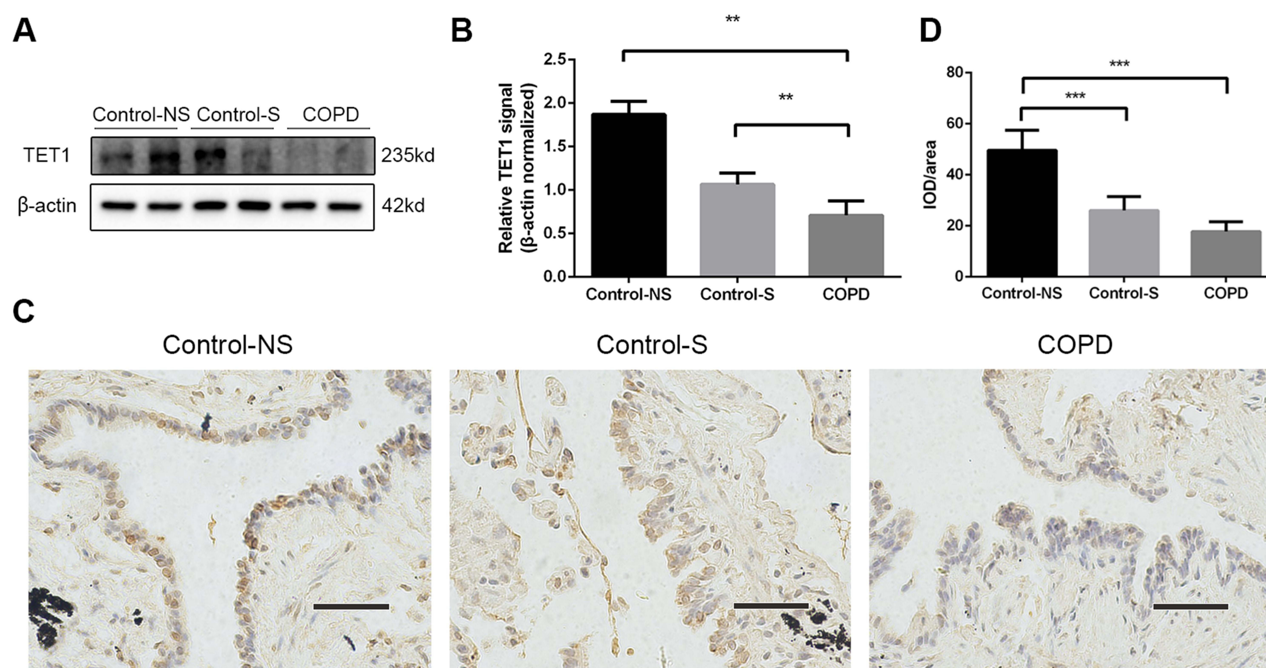

Figure 5 TET1 levels were decreased in the lungs of COPD patients. (**A** and **B**) Western blots of human lung homogenates from the Control-NS (n=9) and Control-S groups (n=6) and COPD patients (n=7) were probed using anti-TET1 antibody; the values were normalized to β-actin levels. (**C**) Immunohistochemical (IHC) staining of TET1 in lung tissues from the Control-NS (n=3) and Control-S (n=3) groups and COPD patients (n=4) (original magnification ×400). The results were scored by (**D**) IOD/area. Scale bars: 50 µm. **P < 0.01; ***P < 0.001.

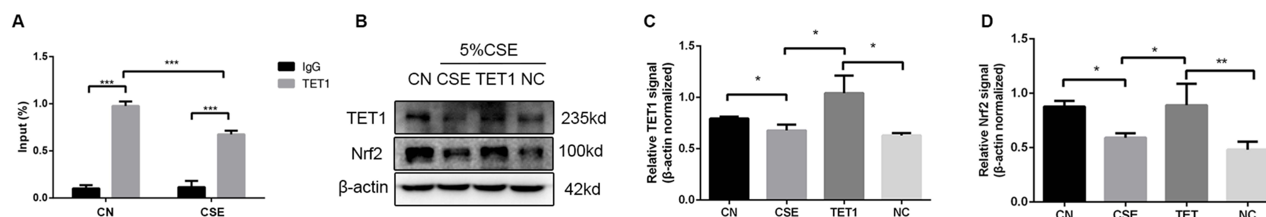

Figure 6 TET1 upregulated Nrf2 by binding the Nrf2 promoter in CSE-treated HBE cells. (**A**) A ChIP assay was carried out by using an anti-TET1 antibody and primers to amplify the Nrf2 promoter region, and the Nrf2 promoter level was quantified. Input represents the amplified total DNA from whole-cell lysates. (n=3) (**B-D**) HBE cells were transfected with TET1 plasmid or vector (NC) and then stimulated with 5% CSE. (**B**) Western blots were probed using (**C**) anti-TET1 antibody or (**D**) anti-Nrf2 antibody; the values were normalized to β-actin levels. (n=3) *P<0.05; **P < 0.01; ***P < 0.001.

Cigarette smoke is a major cause of COPD. Nrf2 plays a protective role against COPD through the activation of antioxidants in the lung. The Nrf2 level was found to be decreased in lung tissues and alveolar macrophages obtained from aged smokers and COPD patients in multiple human studies.[19,20] We found that Nrf2 protein levels were significantly decreased in the peripheral lung, especially in primary bronchial epithelial cells of patients with COPD. In case of oxidative stress, Nrf2 detaches from Keap1 and translocates to the nucleus, where it heterodimerizes with one of the small Maf proteins. The heterodimers recognize the AREs, that are enhancer sequences present in the regulatory regions of Nrf2 target genes, essential for maintaining the oxidation/ antioxidant balance, regulating the expression of a number of cytoprotective genes, such as GPX, HO-1, and NQO1, and taking critical roles in apoptosis, ferroptosis, and other programmed cell death.[21,22] Several studies confirmed that HO-1 protects cells by diminishing oxidative stress and inflammation, and maintaining mitochondrial integrity, thereby promoting cell survival.[23] Nrf2 protein levels were negatively correlated with oxidative injury and apoptosis in HBE cells exposed to CSE. Although our study demonstrated a decrease in Nrf2 expression in CSE-treated HBE cells, we acknowledge that mechanism of nuclear translocation of

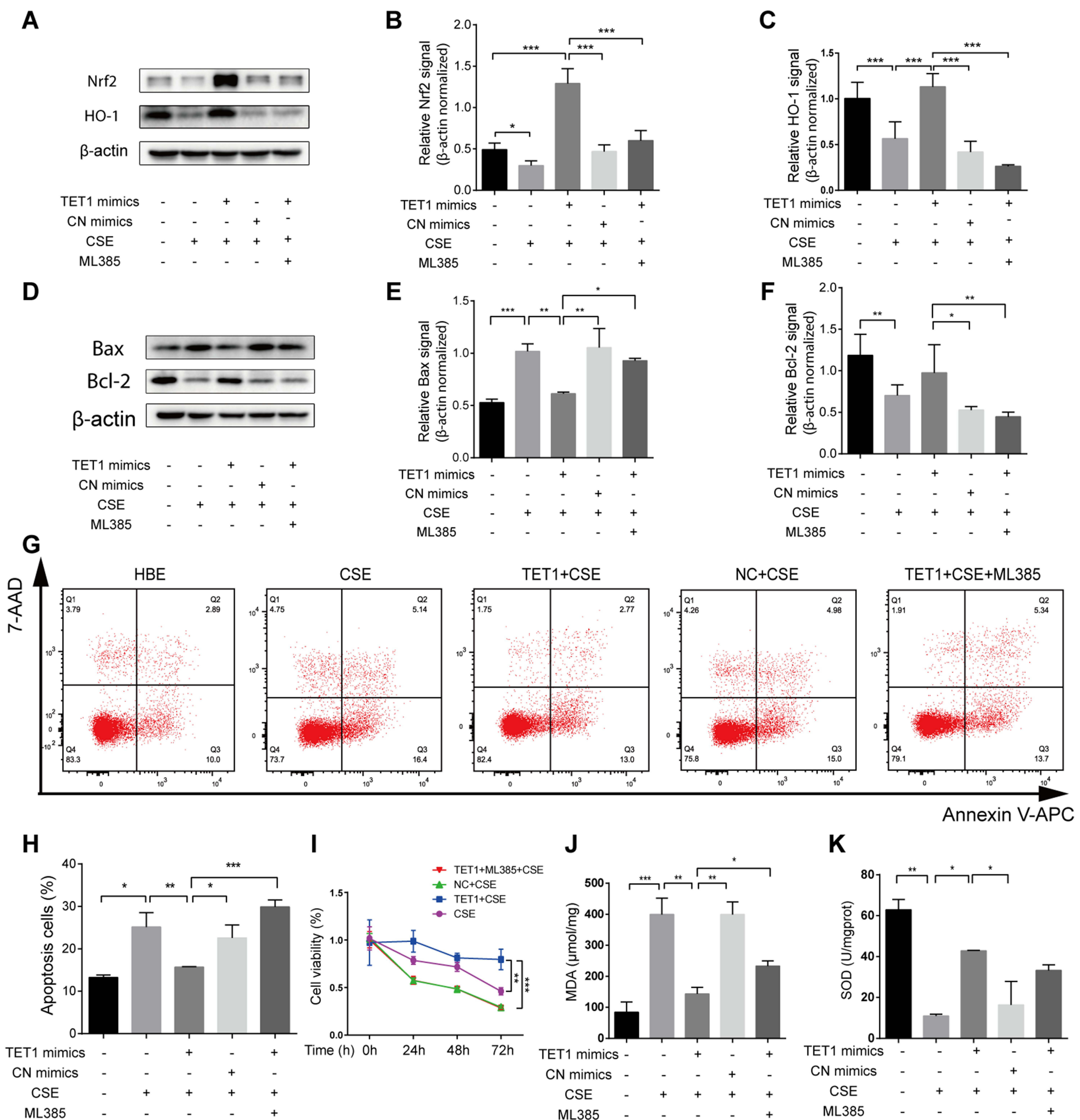

Figure 7 TET1 alleviated apoptosis by upregulating Nrf2. HBE cells were treated with 5% CSE for 72 h. The TET1 plasmid or a control plasmid (NC) was transfected into HBE cells with or without ML385. (**A-C**) Western blots of HBE cells were probed using (**A**) anti-Nrf2 or (**C**) anti-HO-1 antibodies; the values were normalized to β-actin levels. (n=3) (**D-F**) Western blots of HBE cells were probed using (**E**) anti-Bax or (**F**) anti-Bcl-2 antibodies; the values were normalized to β-actin levels. (n=3) (**G** and **H**) Apoptotic HBE cells were detected using Annexin V-APC/7-AAD staining and flow cytometry. (n=3) (**I**) The cell viability of each group was analyzed by the CCK-8 assay, and all values were normalized to those of the control group. (n=3) (**J**) MDA levels and (**K**) SOD activity in each group. (n=3) *P<0.05; **P < 0.01; ***P < 0.001.

Nrf2 under oxidative stress conditions may also contribute to its functional dysregulation. Further studies assessing the localization of Nrf2 in cells are warranted to clarify this mechanism.

It is well established that DNA methylation affects gene expression.[24–26] Our previous study found that CpG hypermethylation led to the suppression of Nrf2 in the lung tissues of COPD patients.[16] In this study, CSE exposure resulted in increased methylation of the Nrf2 promoter in HBE cells at multiple CpG sites, which may negatively correlate with a decrease in Nrf2 levels.

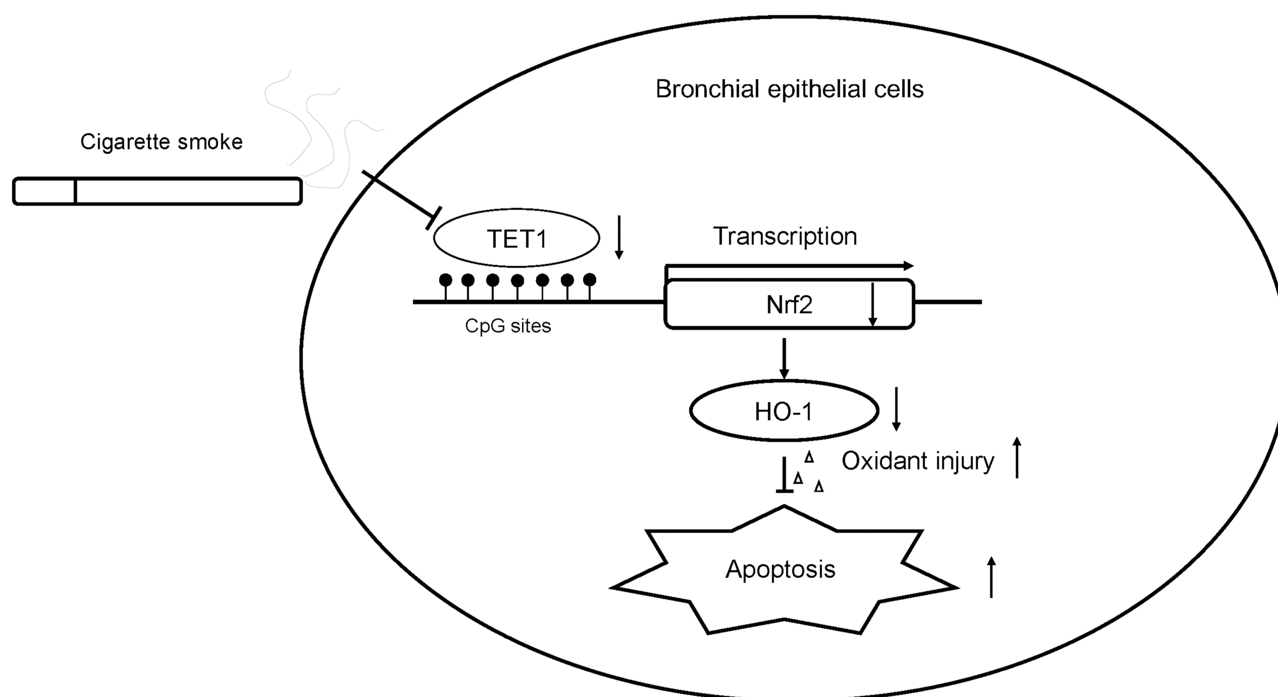

Figure 8 Cigarette smoke down-regulates the level of TET1 in bronchial epithelial cells, which could bind the promoter of Nrf2, leading to hypermethylation of the Nrf2 promoter. Nrf2 level and downstream targets are decreased, the balance of oxidation/antioxidant is damaged, inducing cell apoptosis.

DNMTs transfer a methyl group from S-adenosyl methionine (SAM) to the cytosine of DNA, thus inducing hypermethylation. However, only DNMT1, DNMT3A, and DNMT3B showed activity of methyltransferase. DNMT1 contributes to maintain proper methylation, while DNMT3A and DNMT3B are involved in de novo methylation during development.[27] Remarkably, TET1 was shown to have the opposite effect in erasing DNA methylation by modifying methylcytosine.[28] In this study, we detected DNMT1 and TET1 in vitro to explore the molecular mechanisms of Nrf2 methylation. The results revealed that DNMT1 expression in HBE cells exposed to CSE was not significantly different from that in control HBE cells, whereas TET1 expression was reduced in CSE-treated HBE cells. We further demonstrate that TET1 protein levels in the lung were decreased in patients with COPD compared with subjects without COPD in vivo. It was previously reported that TET1 may play a role in demethylation by binding the CpG islands in the promoter domain.[29] We then determined using a ChIP assay that TET1 could bind the CpG-rich site in the promoter of Nrf2 and upregulate Nrf2, probably due to demethylation of the Nrf2 promoter, alleviating the increased apoptosis of HBE cells treated with CSE. ML385 as a probe molecule that binds to Nrf2 and inhibits its downstream target gene expression.[30] TET1 overexpression enhanced Nrf2 and alleviated apoptosis and oxidant stress, while ML385 reversed the effect in CSE-exposed HBE cells. Nrf2 also plays a critical role in anabolic cancer metabolism by altering glucose and glutamine metabolism.[31,32] Glutaminase inhibitors could be used to determine the effect of TET1 and Nrf2 on glutamine metabolism and metabolic reprogramming changes in COPD.

Further studies should focus on the effect of TET1 on the methylation of the Nrf2 promoter. Additional clinical samples should be obtained and used to detect the regulatory relationship between the TET1 level and lung function. The use of primary bronchial epithelial cells derived from COPD patients would be to better simulate disease-relevant conditions comparing to HBE cells, considering its limitations in fully recapitulating the chronic pathological features of COPD. Furthermore, animal models should be used to explore the role of TET1/Nrf2 in COPD.

In conclusion, reduced expression of TET1 and Nrf2 was observed in lung tissue from patients with COPD. Our findings indicate that CSE exposure induces hypermethylation of the Nrf2 promoter in HBE cells, contributing to oxidative stress and apoptosis. TET1 may bind to the Nrf2 promoter and enhance its transcription, thereby alleviating oxidative stress and protecting HBE cells from apoptosis induced by CSE. These results highlight a potential epigenetic mechanism underlying COPD pathogenesis and suggest that targeting the TET1/Nrf2 may offer a novel therapeutic strategy.

Abbreviations

Nrf2, nuclear factor E2-related factor 2; COPD, chronic obstructive pulmonary disease; CSE, cigarette smoke extract; ROS, reactive oxygen species; ARE, antioxidant response element; HO-1, heme oxygenase-1; CpG islands, CG-rich regions; DNMTs, DNA methyltransferases; TET1, Tet Methylcytosine Dioxygenase 1.

Ethics Approval and Consent to Participate

All experimental work was approved by the Ethical Review Board of Wuxi People's Hospital Affiliated with Nanjing Medical University. This study was conducted in accordance with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, or interpretation or in all these areas. All authors took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that there are no competing interests related to this work.

References

1. de Marco R, Accordini S, Marcon A, et al. Risk factors for chronic obstructive pulmonary disease in a European cohort of young adults. *Am J Respir Crit Care Med*. 2011;183(7):891–897. doi:10.1164/rccm.201007-1125OC
2. Toledo-Pons N, Cosio BG, Velasco MD, Casanova C. Chronic obstructive pulmonary disease in non-smokers. *Archivos de bronconeumologia*. 2017;53:45–46. doi:10.1016/j.arbr.2016.11.033
3. Martinez FD. Early-life origins of chronic obstructive pulmonary disease. *New Engl J Med*. 2016;375:871–878. doi:10.1056/NEJMra1603287
4. Gogebakan B, Bayraktar R, Ulaslı M, Oztuzcu S, Tasdemir D, Bayram H. The role of bronchial epithelial cell apoptosis in the pathogenesis of COPD. *Mol Biol Rep*. 2014;41:5321–5327. doi:10.1007/s11033-014-3403-3
5. Bodas M, Van Westphal C, Carpenter-Thompson R, Mohanty DK, Vij N. Nicotine exposure induces bronchial epithelial cell apoptosis and senescence via ROS mediated autophagy-impairment. *Free Radic Biol Med*. 2016;97:441–453. doi:10.1016/j.freeradbiomed.2016.06.017
6. Prange R, Thiedmann M, Bhandari A, et al. A Drosophila model of cigarette smoke induced COPD identifies Nrf2 signaling as an expedient target for intervention. *Aging*. 2018;10:2122–2135. doi:10.18632/aging.101536
7. Liu Q, Gao Y, Ci X. Role of Nrf2 and its activators in respiratory diseases. *Oxid Med Cell Longev*. 2019;2019:1–17.
8. Goven D, Boutten A, Leçon-Malas V, et al. Altered Nrf2/Keap1-Bach1 equilibrium in pulmonary emphysema. *Thorax*. 2008;63(10):916–924. doi:10.1136/thx.2007.091181
9. Blake DJ, Singh A, Kombairaju P, et al. Deletion of Keap1 in the lung attenuates acute cigarette smoke–induced oxidative stress and inflammation. *Am J Respir Cell Mol Biol*. 2010;42:524–536. doi:10.1165/rcmb.2009-0054OC
10. Steckley D, Karajgikar M, Dale LB, et al. Puma is a dominant regulator of oxidative stress induced Bax activation and neuronal apoptosis. *J Neurosci*. 2007;27:12989–12999. doi:10.1523/JNEUROSCI.3400-07.2007
11. Li D, Ueta E, Kimura T, Yamamoto T, Osaki T. Reactive oxygen species (ROS) control the expression of Bcl-2 family proteins by regulating their phosphorylation and ubiquitination. *Cancer Sci*. 2004;95:644–650. doi:10.1111/j.1349-7006.2004.tb03323.x
12. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev*. 2007;87:99–163. doi:10.1152/physrev.00013.2006
13. Jin C, Lu Y, Jelinek J, et al. TET1 is a maintenance DNA demethylase that prevents methylation spreading in differentiated cells. *Nucleic Acids Res*. 2014;42:6956–6971. doi:10.1093/nar/gku372
14. Davletgildeeva AT, Kuznetsov NA, Li N. The role of DNMT methyltransferases and TET dioxygenases in the maintenance of the DNA methylation level. *Biomolecules*. 2024;15:14. doi:10.3390/biom15010014
15. Silva CP, Kamens HM. Cigarette smoke-induced alterations in blood: a review of research on DNA methylation and gene expression. *Exp Clin Psychopharmacol*. 2021;29:116–135. doi:10.1037/pha0000382
16. Vucic EA, Chari R, Thu KL, et al. DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. *Am J Respir Cell Mol Biol*. 2014;50:912–922. doi:10.1165/rcmb.2013-0304OC
17. Zhang Z, Fu C, Liu J, et al. Hypermethylation of the Nrf2 promoter induces ferroptosis by inhibiting the Nrf2-GPX4 axis in COPD. *Int J Chron Obstruct Pulmon Dis*. 2021;16:3347–3362. doi:10.2147/COPD.S340113
18. Dai X, Ren T, Zhang Y, Nan N. Methylation multiplicity and its clinical values in cancer. *Exp rev mol med*. 2021;23:e2.
19. Expression of concern: decline in NRF2-regulated antioxidants in COPD lungs due to loss of its positive regulator, and heightened endoplasmic reticulum stress in the lungs of patients with COPD. *Am J Respir Crit Care Med*. 2014;190:1200. doi:10.1164/rccm.190101200

20. Suzuki M, Betsuyaku T, Ito Y, et al. Down-regulated NF-E2-related factor 2 in pulmonary macrophages of aged smokers and patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol*. 2008;39:673–682. doi:10.1165/rcmb.2007-0424OC
21. Liu W, Zhao Y, Wang G, et al. TRIM22 inhibits osteosarcoma progression through destabilizing NRF2 and thus activation of ROS/AMPK/mTOR/autophagy signaling. *Redox Biol*. 2022;53:102344. doi:10.1016/j.redox.2022.102344
22. Anandhan A, Dodson M, Schmidlin CJ, Liu P, Zhang DD. Breakdown of an ironclad defense system: the critical role of NRF2 in mediating ferroptosis. *Cell Chem Biol*. 2020;27:436–447. doi:10.1016/j.chembiol.2020.03.011
23. Chiang SK, Chen SE, Chang LC. The role of HO-1 and its crosstalk with oxidative stress in cancer cell survival. *Cells*. 2021;11:10. doi:10.3390/cells11010010
24. Portela A, Esteller M. Epigenetic modifications and human disease. *Nature Biotechnol*. 2010;28:1057–1068.
25. Roman T, Aumüller E, Berner C, Haslberger AG. Interaction of hereditary and epigenetic mechanisms in the regulation of gene expression. *Arquivos brasileiros de cardiologia*. 2010;95:436–439. doi:10.1590/s0066-782x2010005000116
26. Guerrero-Bosagna C, Valladares L. Endocrine Disruptors, Epigenetically Induced Changes, and Transgenerational Transmission of Characters and Epigenetic States. 2007.
27. Wirbisky-Hershberger SE, Sanchez OF, Horzmann KA, Thanki D, Yuan C, Freeman JL. Atrazine exposure decreases the activity of DNMTs, global DNA methylation levels, and dnmt expression. *Food Chem Toxicol*. 2017;109:727–734. doi:10.1016/j.fct.2017.08.041
28. Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*. 2009;324:930–935. doi:10.1126/science.1170116
29. Li H, Jiang W, Liu XN, et al. TET1 downregulates epithelial-mesenchymal transition and chemoresistance in PDAC by demethylating CHL1 to inhibit the Hedgehog signaling pathway. *Oncogene*. 2020;39:5825–5838. doi:10.1038/s41388-020-01407-8
30. Singh A, Venkannagari S, Oh KH, et al. Small molecule inhibitor of NRF2 selectively intervenes therapeutic resistance in KEAP1-deficient NSCLC tumors. *ACS Chem Biol*. 2016;11:3214–3225. doi:10.1021/acschembio.6b00651
31. Mitsuishi Y, Taguchi K, Kawatani Y, et al. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell*. 2012;22:66–79. doi:10.1016/j.ccr.2012.05.016
32. Mukhopadhyay S, Goswami D, Adiseshaiah PP, et al. Undermining glutaminolysis bolsters chemotherapy while NRF2 promotes chemoresistance in KRAS-driven pancreatic cancers. *Cancer Res*. 2020;80:1630–1643. doi:10.1158/0008-5472.CAN-19-1363

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