Introduction

Chronic obstructive pulmonary disease (COPD) involves progressive airflow obstruction and airway inflammation and represents one of the leading causes of morbidity and mortality throughout the world (1). Emphysema is a common component of COPD and is characterized by irreversible destruction of alveolar architecture with enlargement of distal airspaces (2). In pulmonary emphysema, the destruction of the alveolar walls adversely affects the essential functions of the ventilatory mechanism. Without the alveolar tissue elastic recoil and radial support properties, alveolar pressures will cause airway collapse during exhalation (2, 3).

Cigarette smoke (CS) is considered to be the primary risk factor for the development of pulmonary emphysema (4). CS contains greater than 1014 free radicals, including reactive oxygen and reactive nitrogen species per puff, which impose a strong oxidative burden resulting in an oxidant/antioxidant imbalance (5). The relative content of mitochondrial DNA (mtDNA), the only organelle DNA in animal cells encoding 13 subunits of the mitochondrial respiratory chain (MRC) complexes, was significantly decreased in heavy smokers (6). Cytochrome c oxidase (COX) is the last enzyme in the MRC (7, 8) and mainly composed of 13 subunits. In mammals, ten subunits are synthesized from nuclear DNA, and three (COX I, II, III) are synthesized from mtDNA. Acute or chronic CS reportedly decreases the expression and activity of mtDNA encoding COX, and thereby has been associated with diverse MRC dysfunction, which induces apoptosis of epithelial and endothelial cells in the lung (9).

Protective Effect of Demethylation Treatment on Cigarette Smoke Extract–Induced Mouse Emphysema Model

Hongliang Zhang1, Ping Chen2,*, Huihui Zeng2, Yan Zhang2, Hong Peng2, Yan Chen2, and Zhihui He2

1Emergency Department, 2Pulmonary Department, Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China

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Abstract. In the present study, we explored the effects of demethylation in a cigarette smoke extract (CSE)-induced mouse emphysema model. Animals were randomly assigned to the control group, CSE group, 5-aza-2′-deoxycytidine (AZA) group, and CSE+AZA group (n = 10 per group). The mitochondrial transcription factor A (mtTFA) promoter methylation increased over 4-fold in the CSE group compared with the control group, which was reversed by AZA. The mtTFA and the cytochrome c oxidase subunit II (COX II) mRNA and protein levels were decreased approximately 3-fold in the CSE group compared with the control group, which was largely restored by AZA. Histological analysis showed that the CSE group exhibited emphysema compared with the control, which was alleviated by AZA. In addition, CSE significantly induced lung cell apoptosis and decreased lung function and lung mitochondrial COX activity, which was mostly restored by AZA. In conclusion, we for the first time provide evidence that demethylation therapy with AZA can effectively improve emphysema, lung function, lung cell apoptosis, and lung mitochondrial COX activity in a CSE-induced mouse emphysema model, which adds fresh insight into the therapeutic potential of demethylating agents in the prevention and treatment of cigarette smoke-induced emphysema.

Keywords: cigarette smoke, emphysema, demethylation, cytochrome c oxidase, mitochondrial transcription factor A
DNA methylation is a major epigenetic modification in humans (10). Aberrant CpG-island methylation in the gene promoter is associated with gene regulation. Aberrant promoter methylation of multiple genes has been detected in CS (11, 12) and associated with increased risk for pulmonary emphysema and COPD (13). Mitochondrial transcription factor A (mtTFA) plays a key role in the regulation of mtDNA replication and its level is proportional to mtDNA. CpG methylation in the human mtTFA promoter inactivates transcriptional activity driven by the mtTFA promoter (14). A previous study reported that mtTFA-knockdown cell clones showed decreased COX expression (15). Based on all the previous findings, we hypothesized that CS would induce methylation and inactivation of the mtTFA promoter and thereby decrease mtTFA expression, which in turn would lead to decreased COX expression and subsequent MRC dysfunction and epithelial and endothelial cell apoptosis in the lung. In the present study, we tested this hypothesis by exploring the effects of demethylation on lung function, lung cell apoptosis, lung mitochondrial COX activity, and mtTFA and COX II expression in a cigarette smoke extract (CSE)-induced mouse emphysema model.

Materials and Methods

Animals and reagents
Male inbred BALB/c mice (6–8-week-old) were purchased from Central South University (Changsha, China) and were housed at the Xiangya Hospital BioResources Centre. Animals were placed in a quiet, temperature (22°C ± 2°C) and humidity (60% ± 6%) controlled room with a 12:12-h light–dark cycle (light beginning at 8 a.m.), and all tests were performed at the light phase of the cycle. Anti-COX II (N-20) (sc-23983) and anti-mtTFA (F-6) (sc-166965) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). 5-Aza-2′-deoxycytidine (AZA) and all chemicals of reagent grade were purchased from Sigma (St. Louis, MO, USA).

Preparation and treatment with CSE
The CES-treated mouse emphysema model was established as previously described (16). Non-filtered research reference cigarettes (1R3F cigarettes) were purchased from the University of Kentucky in Lexington, KY, USA. CSE was prepared at a concentration of 1 cigarette per 2 ml of phosphate-buffered saline (PBS), which was defined as 100% CSE, and was used after adjusting the pH to 7.4 and filtering through a 0.22-μm filter. Animals were randomly assigned to four groups (n = 10 per group): control group (intraperitoneal injection of PBS), CSE group (intraperitoneal injection of CSE at 0.3 ml·20 g⁻¹), AZA group (intraperitoneal injection of AZA at 2.5 mg·kg⁻¹), and CSE+AZA group (intraperitoneal injection of CSE at 0.3 ml·20 g⁻¹ and AZA at 2.5 mg·kg⁻¹). The treatments were given for 28 days (Table 1). All animals were sacrificed on day 28 after the start of treatment. This study was conducted in accordance with our institutional guidelines on the use of live animals for research, and the experimental protocol was approved by the Laboratory Animal Users Committee at Xiangya Hospital, Central South University, Changsha, China.

Pyrosequencing analysis
Sodium bisulfite conversion of 1 μg of genomic DNA harvested from murine lung tissues was carried out with the EZ DNA Methylation-Direct Kit (Zymo Research, Orange, CA, USA) according to the manufacturer’s protocol. PCR amplification of the lower DNA strand was conducted in a 20-μL reaction volume containing 1 × Coral buffer (QIAGEN, Valencia, CA, USA), 250 nM deoxynucleotides, 250 nM each of forward and reverse biotinylated primers, 1 U of HotStarTaq Plus DNA polymerase (QIAGEN), and 1 μL (50–100 ng) of bisulfite-treated DNA. Amplification conditions were as follows: one cycle at 95°C for 5 min followed by 40 cycles at 95°C for 30 s, 50°C to 52°C for 30 s, 72°C for 30 s, 72°C for 30 s. All treatments were conducted by intraperitoneal injection. All animals were sacrificed on day 28 after the start of treatment. AZA, 5-aza-2′-deoxycytidine; CSE, cigarette smoke extract; PBS, phosphate-buffered saline.

Table 1. Mouse 5-aza-2′-deoxycytidine (AZA) treatment schedule

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>0</th>
<th>11</th>
<th>15</th>
<th>17</th>
<th>19</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
</tr>
<tr>
<td>AZA (2.5 mg/kg)</td>
<td></td>
<td>PBS</td>
<td>PBS</td>
<td>AZA</td>
<td>AZA</td>
<td>AZA</td>
<td>PBS</td>
</tr>
<tr>
<td>CSE (0.3 ml / 20 g)</td>
<td></td>
<td>CSE</td>
<td>CSE</td>
<td>PBS</td>
<td>PBS</td>
<td>PBS</td>
<td>CSE</td>
</tr>
<tr>
<td>CSE+AZA</td>
<td></td>
<td>CSE</td>
<td>CSE</td>
<td>AZA</td>
<td>AZA</td>
<td>AZA</td>
<td>CSE</td>
</tr>
</tbody>
</table>

All treatments were conducted by intraperitoneal injection. All animals were sacrificed on day 28 after the start of treatment. AZA, 5-aza-2′-deoxycytidine; CSE, cigarette smoke extract; PBS, phosphate-buffered saline.
for 30 s, and a final extension step at 72°C for 10 min. Prior to pyrosequencing, PCR products were analyzed by agarose gel electrophoresis/ethidium bromide staining. Pyrosequencing was conducted in a mixture of 5 to 10 μL of PCR product with 2 μL of streptavidin-coated Sepharose beads (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and 40 μL of binding buffer (10 mM Tris-HCl, 2 M NaCl, 1 mM EDTA, 0.1% Tween 20, pH 7.6) brought up to 80 μL total volume with distilled H2O. This mixture was immobilized on streptavidin beads by shaking constantly at 1400 rpm for 10 min at room temperature. The beads were harvested with a vacuum preparation workstation and appropriate sequencing primer added to a final concentration of 330 nM. The sequencing primers were annealed to the template by incubating the samples at 80°C for 2 min and cooled at RT for 10 min prior to analysis. Pyrosequencing was conducted with a PyroMark MD system (QIAGEN) and sequence analysis conducted with the supplied Pyro Q-CpG software by Biotage AB (Uppsala, Sweden).

Lung function measurement

Mouse lung function was measured on day 28 after the start of CSE exposure using an AniRes 2005 mouse lung function analysis system (version 2.0; Bestlab, Beijing, China). Respiratory frequency (f), tidal volume (TV), airway inspiratory resistance (RI), lung dynamic compliance (Cdyn), and peak inspiratory flow (PIF) were measured.

Histological examination

The lungs were fixed in situ for 2 min with buffered formalin (4%) via endotracheal instillation at a pressure of 25 cmH2O. The trachea was blocked, after which the lungs were removed and immersed in fixing solution for 48 h. After the final paraffin-embedding, 5-μm sections were cut and the slides were stained with hematoxylin-eosin, as is the standard procedure. Pulmonary emphysema was quantified based on the degree of alveolar destruction, as determined by measuring the mean linear intercept (Lm, mean alveolar diameter) in micrometers. In brief, this technique consists of the determination of the number of times the gas exchange structures in the parenchyma intersect a series of grid lines. Therefore, in the presence of emphysema, the number of intercepts of the alveolar structures with a system of lines will be fewer, indicating alveolar destruction. The Lm is obtained using the equation Lm = Ltot/Li, where Ltot is the total length of the lines in the microscopic field, and Li is the number of intercepts of alveolar structures with the lines of the reticulum. To that end, 16 fields of each slide were counted and observed at a magnification of × 200 through a reticulum attached to the monitor. Stereology was carried out using a test-system composed of 21 points and in a known test area delineated by the forbidden line, in order to avoid overestimation of the number of structures. The test system was attached to a monitor connected to a microscope. The points (PP) that intercepted the cFs and the eFs were counted and compared with the total number of points of a test system (TP). Therefore, the volume density (Vv) is equal to the PP divided by the TP (17).

Lung cell apoptosis measurement

For the TdT-mediated dUTP nick end labeling (TUNEL) assay, histopathology slides were rehydrated in PBS for 10 min before boiling in citrate buffer (100 mM citrate, pH 6.0, 0.05% Tween 20) for 5 min. TUNEL staining was performed using the In Situ Cell Death Detection Kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer’s protocol. Coverslips were applied using mounting solution containing the nuclear stain DAPI, and fluorescence was detected using an Olympus 370 inverted fluorescence microscope. The number of TUNEL (FITC)-positive cells per high-power field (magnification × 3400) was determined after acquisition of images using a charge-coupled device camera and Metamorph software.

Real-time quantitative reverse transcription PCR

RNA was prepared from lung tissues using TRIzol reagent followed by purification with the TURBO DNA-free System (Ambion, Austin, TX, USA). The cDNAs were synthesized using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR was performed on the LightCycler thermal cycler system (Roche Diagnostics) using SYBR Green I kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer’s protocol. Coverslips were applied using mounting solution containing the nuclear stain DAPI, and fluorescence was detected using an Olympus 370 inverted fluorescence microscope. The number of TUNEL (FITC)-positive cells per high-power field (magnification × 3400) was determined after acquisition of images using a charge-coupled device camera and Metamorph software.

Western blot analysis

Immunoblotting was performed with the respective antibodies. Briefly, lung tissues were homogenized and lysed in 250 μl of 2 × SDS loading buffer (62.5 mm TrisHCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% brom-
phenol blue, 5% 2-mercaptoethanol) and incubated at 95°C for 10 min. Equal amount of protein for each sample was separated by a 10% SDS-polyacrylamide gel and then blotted onto a polyvinylidene difluoride microporous membrane (Millipore, Billerica, MA, USA). Membranes were incubated for 1 h with a 1/1000 dilution of primary antibody, washed, and protein bands were revealed using horseradish peroxidase–conjugated secondary antibodies (1/5000, 1 h) and the GE Healthcare ECL kit. Proteins were quantified before being loaded onto the gel, and equal loading of extracts was verified by Ponceau coloration.

**Measurement of lung mitochondrial COX activity**

Mouse lungs were extracted, and lung mitochondria were obtained using a mitochondria isolation kit (Sigma). In brief, fresh lungs were washed and homogenized with a rotor-stator homogenizer (T10 basic Ultra-Turrax®; IKA, Staufen, Germany) at 15,000 rpm in extraction reagent. The homogenate was centrifuged at 600 × g for 5 min, and then the supernatant was further centrifuged at 11,000 × g for 10 min. The pellet was suspended in storage buffer and used as a mitochondrial fraction. Protein concentrations were determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). The COX activity in lung mitochondria was measured using a cytochrome c oxidase assay kit (Sigma). In brief, 5 μg of the mitochondrial fraction were diluted with enzyme dilution buffer containing 1 mM n-dodecyl β-d-maltoside. Ferrocytochrome c (reduced cytochrome c with dithiothreitol) was then added to the sample, and COX activity was measured by the decrease in absorption at 550 nm. The difference in extinction coefficients between reduced and oxidized cytochrome c is 21.84 at 550 nm. One unit of COX activity was defined as the oxidation of 1.0 μmol of ferrocytochrome c per min at pH 7.0 at 25°C.

**Statistical analyses**

Statistical analyses were performed with SPSS for Windows 10.0. Data values were expressed as means ± S.D. Comparisons of means among multiple groups were performed with one-way ANOVA followed by post hoc pairwise comparisons using the least significant difference method. The significance level of this study was set at a two-tailed α = 0.05.

**Results**

As shown in Fig. 1, the mtTFA promoter methylation increased over 4-fold in the CS group compared with the Control and the AZA groups, which was reversed by AZA treatment. Real-time RT-PCR showed that the mtTFA and the COX II mRNA levels were decreased approximately 3-fold in the CSE group compared with the Control and the AZA groups, which was largely restored by AZA treatment (Fig. 2). Similar results were observed at the mtTFA and the COX II protein levels in western blot analyses (Fig. 3). As shown in Fig. 4, histological image analyses showed that the CSE group exhibited obvious emphysema compared with the Control and the AZA groups. AZA demethylation treatment alone resulted in no significant change compared with the control. However, in the CSE+AZA group, AZA significantly alleviated the CSE-induced emphysema (Fig. 4).

Compared with the control, CSE showed significantly negative impact on TV, RI, Cdyn, and PIF, which was reversed by AZA (Table 2). Lung cell apoptosis analyses showed that compared with the Control group, the CSE group showed marked increase in total apoptotic cells in the lung (including a variety types of cells such as alveolar epithelial cells and lung vascular endothelial cells), which was significantly alleviated by AZA (Fig. 5, Table 3).

As shown in Table 4, CSE significantly decreased lung mitochondrial COX activity compared with the control; AZA treatment largely restored the decreased COX activity in the lung, although AZA alone showed no significant effect on the COX activity.

**Discussion**

In the present study, we explored the effects of demethylation on lung function, lung cell apoptosis, lung
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Fig. 2. Mitochondrial transcription factor A (mtTFA) and cytochrome c oxidase subunit II (COX II) mRNA levels in experimental groups. The mtTFA (A) and the COX II (B) mRNA levels in the Control group, 5-aza-2′-deoxycytidine (AZA) group, cigarette smoke extract (CSE) group, and CSE+AZA group (n = 10 in duplicate in each group) were analyzed with real-time RT-PCR. aP < 0.05 vs. CSE, bP < 0.05 vs. AZA, cP < 0.05 vs. CSE+AZA.

Fig. 3. Mitochondrial transcription factor A (mtTFA) and cytochrome c oxidase subunit II (COX II) protein levels in experimental groups. A) The mtTFA and the COX II protein levels in the Control group, 5-aza-2′-deoxycytidine (AZA) group, the cigarette smoke extract (CSE) group, and the CSE+AZA group (n = 10 in each group) were analyzed with western blot analysis. β-Actin blotting was used as a loading control. Density of mtTFA (B) and COX II (C) was normalized against that of β-actin to obtain a relative blot density, which was expressed as fold change to the relative blot density of the Control (designated as 1). aP < 0.05 vs. CSE, bP < 0.05 vs. AZA, cP < 0.05 vs. CSE+AZA.

Table 2. Lung function in experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>F (bpm)</th>
<th>TV (mL)</th>
<th>RI (cmH₂O·mL⁻¹·min⁻¹)</th>
<th>Cdyn (mL/cmH₂O)</th>
<th>PIF (mL/s)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>185.666 ± 3.502</td>
<td>0.459 ± 0.006</td>
<td>0.069 ± 0.005</td>
<td>0.217 ± 0.010</td>
<td>5.521 ± 0.099</td>
</tr>
<tr>
<td>AZA (2.5 mg/kg, IP injection)</td>
<td>184.166 ± 3.601</td>
<td>0.443 ± 0.007</td>
<td>0.075 ± 0.009*</td>
<td>0.209 ± 0.005*</td>
<td>5.147 ± 0.049</td>
</tr>
<tr>
<td>CSE</td>
<td>183.166 ± 2.927</td>
<td>0.375 ± 0.008*</td>
<td>0.212 ± 0.012*</td>
<td>0.109 ± 0.006*</td>
<td>4.310 ± 0.051*</td>
</tr>
<tr>
<td>CSE+AZA</td>
<td>183.500 ± 2.739</td>
<td>0.443 ± 0.006*</td>
<td>0.072 ± 0.005*</td>
<td>0.162 ± 0.006*</td>
<td>5.199 ± 0.076*</td>
</tr>
</tbody>
</table>

Mouse lung function was measured 30 days after the start of CSE exposure. AZA, 5-aza-2′-deoxycytidine; CSE, cigarette smoke extract; PBS, phosphate-buffered saline. F, respiratory frequency; TV, tidal volume; RI, airway inspiratory resistance; Cdyn, lung dynamic compliance; PIF, peak inspiratory flow; IP, intraperitoneal; *P < 0.05 vs. Control; †P < 0.05 vs. CS.
Fig. 4. Histological images of lungs in experimental groups. A, Control; B, 5-aza-2′-deoxycytidine (AZA, 2.5 mg/kg, intraperitoneal injection); C, cigarette smoke extract (CSE); D, CSE+AZA; E, mean linear intercept in each experimental group (n = 10). Magnification, ×40. *P < 0.05 vs. CSE, †P < 0.05 vs. AZA, ‡P < 0.05 vs. CSE+AZA.

Fig. 5. Immunofluorescence images of lung cell apoptosis in experimental groups. A, Control; B, 5-aza-2′-deoxycytidine (AZA, 2.5 mg/kg, intraperitoneal injection); C, cigarette smoke extract (CSE); D, CSE+AZA; E, positive-staining control; F, negative-staining control.
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Table 3. Lung cell apoptosis rates in experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Apoptosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>AZA (2.5 mg/kg, IP injection)</td>
<td>10</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>CSE</td>
<td>10</td>
<td>14.2 ± 0.6*</td>
</tr>
<tr>
<td>CSE+AZA</td>
<td>10</td>
<td>8.1 ± 0.5*#</td>
</tr>
</tbody>
</table>

AZA, 5-aza-2′-deoxycytidine; CSE, cigarette smoke extract; IP, intraperitoneal; *P < 0.05 vs. Control; #P < 0.05 vs. CS.

Table 4. Lung mitochondrial cytochrome c oxidase (COX) activity in experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>COX activity (× 10⁻¹ U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>AZA (2.5 mg/kg, IP injection)</td>
<td>10</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>CSE</td>
<td>10</td>
<td>4.2 ± 0.4*</td>
</tr>
<tr>
<td>CSE+AZA</td>
<td>10</td>
<td>5.1 ± 0.5*#</td>
</tr>
</tbody>
</table>

AZA, 5-aza-2′-deoxycytidine; CSE, cigarette smoke extract; IP, intraperitoneal; *P < 0.05 vs. Control; #P < 0.05 vs. CS.

mitochondrial COX activity, and mtTFA and COX II expression in a CSE-induced mouse emphysema model.

The CSE-induced emphysema model used in the present study has been well established in both rodents and reported in several previous studies (16–19). Intraperitoneal CSE injection caused enlargement of the alveolar air spaces in rats by 35% (17, 18) and this model was also replicated in mice (16, 19). The mechanism in this novel animal emphysema model is not completely known but thought to be involved with an autoimmune mechanism in alveolar septal cell destruction (20, 21). It is unclear whether all pathophysiologically relevant mechanisms in this model are shared with the conventional model in which CS is inhaled over a period of several months. Nevertheless, following intraperitoneal injection of CSE for 3 weeks, mice exhibited pulmonary emphysema pathologically with airspace enlargement and alveolar destruction.

AZA is a nucleoside analog that can inhibit DNA cytosine methylation by incorporating into DNA and inhibiting DNA (cytosine-5)-methyltransferases (22). Before the present study, we performed a pilot study to determine the delivery method and drug concentration of AZA for demethylation treatment and found that intraperitoneal injection of AZA at 2.5 mg·kg⁻¹ would generate the optimal therapeutic effects with minimal side effects. Indeed, AZA delivered this way reversed CSE-induced methylation of the mtTFA promoter and restored the lung function damaged by CSE in the present study.

DNA methylation may provide an explanation for features of pulmonary emphysema or COPD that are not explained fully by DNA sequence variation, such as the variable susceptibility to develop lung disease in smokers, as well as the continued elevated risk for lung function decline years after smoking cessation (23). CS is a major environmental risk factor for lung function decline and has been shown to impact the methylation patterns of individual genes (23). In the present study, we showed that CSE could induce methylation of the mtTFA promoter, which would turn off the promoter activity (14). In agreement with the results, the mtTFA expression decreased at both the mRNA and the protein levels after CSE exposure and could be largely restored by demethylation with AZA. In line with a recent report that COX II was regulated by mtTFA (15), our results showed that the COX II expression as well as the lung mitochondrial COX activity decreased along with the decreased mtTFA expression. COX II is a major component of MRC, whose deficiency will result in epithelial and endothelial cell apoptosis in the lung (9). Indeed, our results showed that the decreased mtTFA and COX II expression resulted from CSE-induced mtTFA promoter methylation led to decreased mitochondrial COX activity and increased cell apoptosis and emphysema in the lung. Based on the findings, we have confirmed our hypothesis that CSE induces methylation and inactivation of the mtTFA promoter and thereby decrease mtTFA expression, which in turn leads to decreased COX II expression and subsequent MRC dysfunction and epithelial and endothelial cell apoptosis in the lung. However, since CS can impact global methylation of genomic elements (24, 25), the mtTFA-COX II axis is only one of too many affected by CS-induced methylation. Further studies are needed to uncover other pathways or mechanisms involved. The major point of this study is that demethylation treatment with AZA can effectively reverse the lung function damaged by CSE, which suggests a new paradigm for prevention and treatment of CS-induced emphysema.

In conclusion, we for the first time provide evidence that demethylation therapy with AZA can effectively improve emphysema, lung function, lung cell apoptosis, and lung mitochondrial COX activity in a CSE-induced mouse emphysema model, which adds fresh insight into the therapeutic potential of demethylating agents in prevention and treatment of CS-induced emphysema.

Acknowledgments

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Conflicts of Interest

The authors declare that they have no competing interest.

References