

Taurine ameliorates particulate matter-induced emphysema by switching on mitochondrial NADH dehydrogenase genes

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Chronic obstructive pulmonary disease (COPD) has been linked to particulate matter (PM) exposure. Using transcriptomic analysis, we demonstrate that diesel exhaust particles, one of the major sources of particulate emission, down-regulated genes located in mitochondrial complexes I and V and induced experimental COPD in a mouse model. 1-Nitropyrene was identified as a major toxic component of PM-induced COPD. In the panel study, COPD patients were found to be more susceptible to PM than individuals with normal lung function due to an increased inflammatory response. Mechanistically, exposure to PM in human bronchial epithelial cells led to a decline in CCAAT/enhancer-binding protein alpha (C/EBP α), which triggered aberrant expression of NADH dehydrogenase genes and ultimately led to enhanced autophagy. ATG7-deficient mice, which have lower autophagy rates, were protected from PM-induced experimental COPD. Using metabolomics analysis, we further established that treatment with taurine and 3-methyladenine completely restored mitochondrial gene expression levels, thereby ameliorating the PM-induced emphysema. Our studies suggest a potential therapeutic intervention for the C/EBP α /mitochondria/autophagy axis in PM-induced COPD.

particulate matter | chronic obstructive pulmonary disease | mitochondria | autophagy | taurine

Chronic obstructive pulmonary disease (COPD), one of the leading causes of death worldwide (1), is characterized by emphysema and small airway wall remodeling (2). The pathogenesis of COPD is related to chronic environmental stress and is associated with inflammatory host immune response (3). Cigarette smoke is the most common irritant that causes COPD; however, at least one-fourth of patients with COPD are nonsmokers; thus the disease may be attributed to air pollution, chemical fumes, or dust exposure (4). In China, severe air pollution has become a major threat to public health, exacerbating the detrimental effects on respiratory health. It is noteworthy that in certain highly polluted regions daily concentrations of particulate matter (PM) commonly reach levels in the range of 150–500 $\mu\text{g}/\text{m}^3$ (5).

The respiratory system is a primary target of inhaled PM. Although the relationship between air pollution and pulmonary function is established (6), considerable variability exists in the reduction in pulmonary function of patients following exposure to air pollution containing diverse components (7, 8). Diesel exhaust particles (DEP), one of the major sources of PM emission, are typically composed of a carbonaceous core coupled with a complex mixture of more than 40 toxic adsorbed components (9). DEP exposure results in the reduction of mitochondrial biogenesis, oxidative phosphorylation, and ATP synthesis, leading to increased oxidative stress as well as inflammatory responses, all of which play important roles in the pathogenesis of COPD (10, 11). Furthermore, mitochondrial dysfunction and

enhanced oxidative stress are capable of triggering an essential degradation process known as autophagy (12).

The role of autophagy in pulmonary disorders can be either deleterious or protective, depending on the stimuli (13). In cigarette-smoke-induced COPD, autophagy is critical in mediating apoptosis and cilia shortening in airway epithelia (14, 15). Autophagy, in turn, accelerates lung aging and emphysema and contributes to COPD pathogenesis by promoting epithelial cell death (16, 17). An autophagy-related pathway has been identified as essential for airborne ultrafine particle-induced inflammation and pulmonary damage both in vitro and in vivo (18). However, the critical physiological functions of autophagy in the pathogenesis of PM-induced COPD have yet to be delineated.

Here, we investigated changes in gene expression profiles in human bronchial epithelial (HBE) cells and COPD-like lesions in mouse models upon exposure to DEP to evaluate the molecular pathogenesis associated with air-pollution-induced COPD. Furthermore, we undertook a panel study to ascertain which component of

Significance

Exposure to high levels of particulate matter (PM) poses a major threat to human health. Cigarette smoke is the most common irritant that causes chronic obstructive pulmonary disease (COPD); however, at least one-fourth of patients with COPD are nonsmokers, and their disease is largely attributed to air pollution. The occurrence of pollution episodes in China has raised an emergent question of how PM leads to the pathogenesis of COPD. In this paper, we show that deregulation of mitochondrial NADH dehydrogenase gene expression levels plays a key role in the aggravation of COPD during air pollutant exposure, which can be rescued by taurine and 3-MA treatments in both mammalian cells and animals.

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Data deposition: The microarray datasets reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, <https://www.ncbi.nlm.nih.gov/geo> (accession no. [GSE82335](https://www.ncbi.nlm.nih.gov/geo/acc/show?acc=GSE82335)).

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DEP best associated with COPD. Notably, we also show that switching on mitochondrial NADH dehydrogenase genes alleviates experimental COPD, highlighting a potential therapeutic target for PM-induced COPD.

Results

Overview of mRNA Microarray Profiles. Airway epithelial cells are the primary defense of organisms against inhaled pathogen and chemicals. Therefore, the mRNA of HBE cells was evaluated with a RNA microarray to determine alterations in gene transcription in response to DEP exposure. DEP exposure exerted specific transcriptional effects leading to a significantly increased expression of 313 genes and a decreased expression of 489 genes, with a cutoff of two or more fold changes (FCs) and $P < 0.05$ (*SI Appendix, Fig. S1A*). A gene ontology (GO) enrichment analysis classified transcriptional differences between the control and treatment groups. Notably, genes encoding proteins necessary for mitochondrial function were differentially expressed in the two groups (*SI Appendix, Fig. S1 B–D*).

Then the down-regulated genes were annotated with the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. The hypergeometric test was used to determine the significance of the overrepresentation; oxidative phosphorylation was the most significantly enriched pathway (*SI Appendix, Fig. S1E and Tables S1 and S2*). The NADH ubiquinone dehydrogenase-encoding genes (i.e., *NDUFA2* and *NDUFS4*), the NADH dehydrogenase-encoding genes located in mitochondrial complex I (*NDUFC2* and *NDUFA1*), and the mitochondrial ATP synthase gene *ATP5H* (complex V, F0 unit) were identified by both GO and KEGG enrichments as the most significantly altered in response to DEP exposure.

Modulations of mRNA Expression Confirmed by Quantitative Real-Time PCR. We performed quantitative real-time PCR (qRT-PCR) analysis to validate our RNA microarray data and assessed the top 10 differentially expressed mitochondria-related genes. In HBE cells and lung tissues obtained from mice treated with DEP, *RAC1*, *COX18*, and *SH3GLB1* were up-regulated; and *NDUFA1*, *NDUFA2*, *NDUFS4*, *NDUFC2*, *ATP5H*, *PRDX2*, and *NOXO1* were down-regulated in a dose-dependent manner (*SI Appendix, Fig. S2 A–D*). With the exception of *RAC1*, which was confirmed to be up-regulated by qRT-PCR analysis, mRNA regulation trends for the other nine genes were consistent between the microarray and the qRT-PCR (*SI Appendix, Fig. S2 E and F*). Therefore, both bioinformatics analysis and biological assays suggested an association between DEP exposure and mitochondrial dysfunction.

In addition to the aberrant mitochondrial gene expression levels, COPD-like lesions in mouse lung, such as emphysema (destruction of alveolar walls) and small airway remodeling (small airway fibrosis) [all established indices of experimental COPD (19, 20)], as well as alveolitis were observed in the DEP-treated mouse lung tissues (*SI Appendix, Fig. S3*).

1-Nitropyrene Is the Major Toxic Component That Induces Modulation of Mitochondrial Genes. To identify the major toxic components of DEP that affect mitochondria, HBE cells were treated with the most prevalent two nitropolyaromatics (nitro-PAHs) or three PAHs in DEP (*SI Appendix, Table S3*) at concentrations of 1 and 10 $\mu\text{g}/\text{mL}$, respectively. Atmospheric nitro-PAHs can be emitted from incomplete combustion of fossil fuels, especially diesel, or formed through photochemical reactions from parent PAHs (21). RNA regulation trends of *NDUFA1*, *NDUFA2*, *NDUFC2*, *NDUFS4*, and *ATP5H* in response to 1-nitropyrene [(1-NP) the most abundant nitro-PAH component in DEP (22)] were consistent with those noted in DEP-exposed HBE cells (*SI Appendix, Fig. S4A*). The five mitochondria-related gene expression levels were significantly inhibited in murine lung tissues exposed to 1-NP (*SI Appendix, Fig. S4B*). Next, the subcellular effects of 1-NP were

confirmed by transmission electron microscopy analysis in HBE cells. Swollen mitochondria (*SI Appendix, Fig. S4C*, white arrow) and autophagosomes (*SI Appendix, Fig. S4C*, black arrow) were observed in the cytoplasm of 1-NP-treated HBE cells. Our results suggested that, among the five most abundant components of DEP, 1-NP was the major one that triggered mitochondrial inhibition in pulmonary cells.

PM Exposure Increases Levels of Inflammatory Mediators in COPD Patients. To assess the health risk following air pollution exposure, we assessed 1-NP metabolite levels in the urine of subjects from three different Chinese cities and found that the urinary 6-OHNP and 8-OHNP levels were significantly increased in subjects living in cities with moderate air pollution compared with those experiencing better air quality (*SI Appendix, Table S4*). Subsequently, lung function and levels of inflammatory mediators and their association with 1-NP exposure were assessed in healthy individuals and COPD patients. Daily air pollution index and air pollutant concentrations during a consecutive 7-d-observation period are shown in *SI Appendix, Table S5*. Notably, this period was one of the most polluted periods in Nanjing and Shijiazhuang during the 2015 winter season. The mean 1-NP levels collected from filters, creatinine-adjusted 1-NP metabolites in urine, and IL-6 levels in serum are shown in *SI Appendix, Table S6*. Both creatinine-adjusted urinary 6-OHNP and 8-OHNP levels increased in healthy subjects and COPD patients exposed to air pollution, while enhanced IL-6 levels were observed only in the serum of COPD patients. The increase in serum IL-6 levels correlated with increased 1-NP metabolites (6-OHNP and 8-OHNP) in COPD patients (*SI Appendix, Fig. S5 A and B*), but not in healthy subjects (*SI Appendix, Fig. S5 C and D*). Together, the results suggest that mild-to-moderate COPD patients are more susceptible to air pollutants than normal individuals.

Deregulation of Mitochondrial Genes Is Transcriptionally Driven by C/EBP α . The results mentioned above led us to investigate the upstream mechanisms delineating mitochondrial responses to 1-NP exposure. The potential binding sites with transcription factors of *NDUFA1*, *NDUFA2*, *NDUFS4*, *NDUFC2*, and *ATP5H* promoter regions were identified by the University of California at Santa Cruz genome browser (*SI Appendix, Fig. S6A*).

HBE cells and mouse lung tissue were efficiently infected with C/EBP α lentivirus tagged by c-Myc (*Fig. 1A*). Overexpression of C/EBP α rescued the binding of C/EBP α to the *NDUFA1*, *NDUFA2*, *NDUFS4*, *NDUFC2*, and *ATP5H* promoter regions (*Fig. 1B and SI Appendix, Fig. S6 B–E*). *Fig. 1C* shows that 1-NP-induced emphysema was ameliorated by C/EBP α overexpression. Single 1-NP exposure induced moderate-to-severe alveolitis. 1-NP coupled with C/EBP α overexpression treatment led to mild-to-moderate alveolitis in the mouse lung tissue (*SI Appendix, Fig. S7*). Conversely, when C/EBP α expression was inhibited in mouse lungs, the histological lesion, including emphysema and alveolitis, were exacerbated with 1-NP treatment (*SI Appendix, Fig. S8 A–D*).

1-NP cytotoxicity was evaluated using the CCK-8 assay. The growth of HBE cells was inhibited by 1-NP exposure in a concentration-dependent manner (*SI Appendix, Fig. S8E, Left*). Overexpression of C/EBP α completely rescued cell growth to levels indistinguishable from controls within 72 h in the 1- $\mu\text{g}/\text{mL}$ 1-NP-treated group. The cell growth in the 10- $\mu\text{g}/\text{mL}$ 1-NP-treated group was partially rescued by overexpression of C/EBP α within 72 h (*SI Appendix, Fig. S8E, Right*). Our results mentioned above suggest that C/EBP α is an upstream regulator of mitochondrial NADH dehydrogenase genes following 1-NP exposure.

Mitochondrial Complex I Dysfunction Triggers Autophagy in an ATG7-Dependent Manner. As noted above, mitochondrial function conferred important impacts in the cellular response to 1-NP exposure. We therefore sought to delineate the mechanisms by

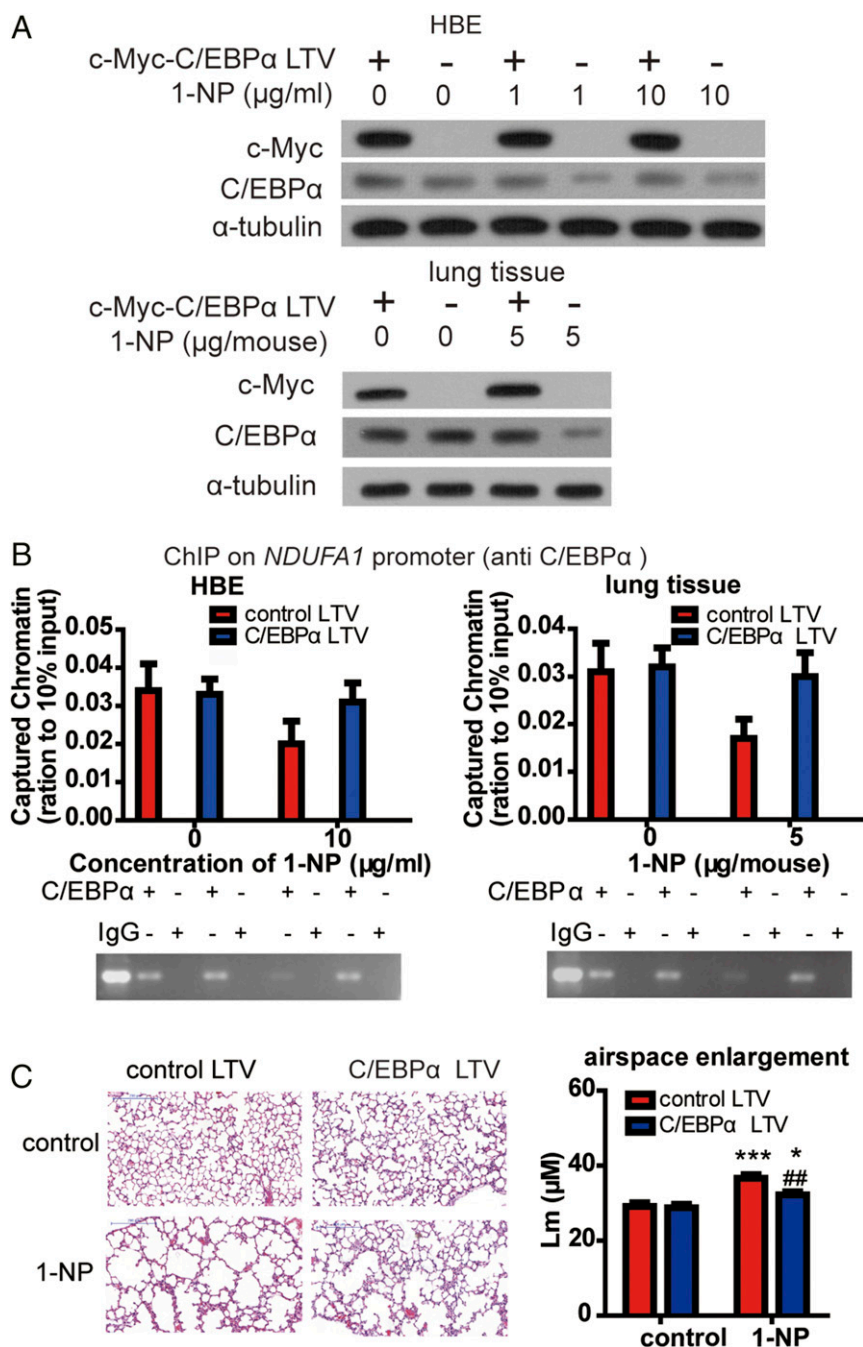


Fig. 1. C/EBP α transcriptionally regulates the expression of oxidative phosphorylation-involved genes. (A) Immunoblot assay of c-Myc expression. (B) ChIP assays suggested that the binding of C/EBP α to *NDUFA1* was affected by 1-NP exposure. (C) C/EBP α overexpression partially rescued 1-NP-induced emphysema in mouse lung tissues (100 \times). (Scale bar: 200 μ m.) * P < 0.05 and *** P < 0.001, compared with control lentivirus (LTV)-treated group; ## P < 0.01, compared with 1-NP and control LTV-treated group (n = 18).

which 1-NP promoted mitochondrial dysfunction. The integrity of the mitochondrial membrane, reactive oxygen species (ROS) generation, and autophagy rates were monitored in HBE cells exposed to 1-NP. The fluorescent probe JC-1 showed red fluorescence in normal cells, indicating intact mitochondrial membrane potential. However, cells treated with 1 or 10 μ g/mL 1-NP showed decreased red and strong green fluorescence, consistent with altered mitochondrial membrane potential (Fig. 2A). ROS levels in HBE cells were detected by fluorescent microscopy and flow cytometry 24 h after exposure and were significantly increased in a concentration-dependent manner (Fig. 2B).

Autophagic pathway-related gene expression levels were detected by qRT-PCR in 1-NP-treated HBE cells and mouse lung tissue, where enhanced expression of *ATG7* and *LC3B* [injury markers previously shown to be associated with emphysema (20, 23)] were observed (Fig. 2C). To further confirm that 1-NP exposure increased autophagy, we assessed *ATG7* and *LC3B* protein levels by Western blot and autophagic flux by Cyto-ID autophagy detection kits. 1-NP exposure increased *ATG7* and *LC3B-II* protein expression, consistent with mRNA expression levels (Fig. 2D). The puncta labeled by Cyto-ID significantly increased, suggesting enhanced autophagosome formation in HBE cells (Fig. 2E).

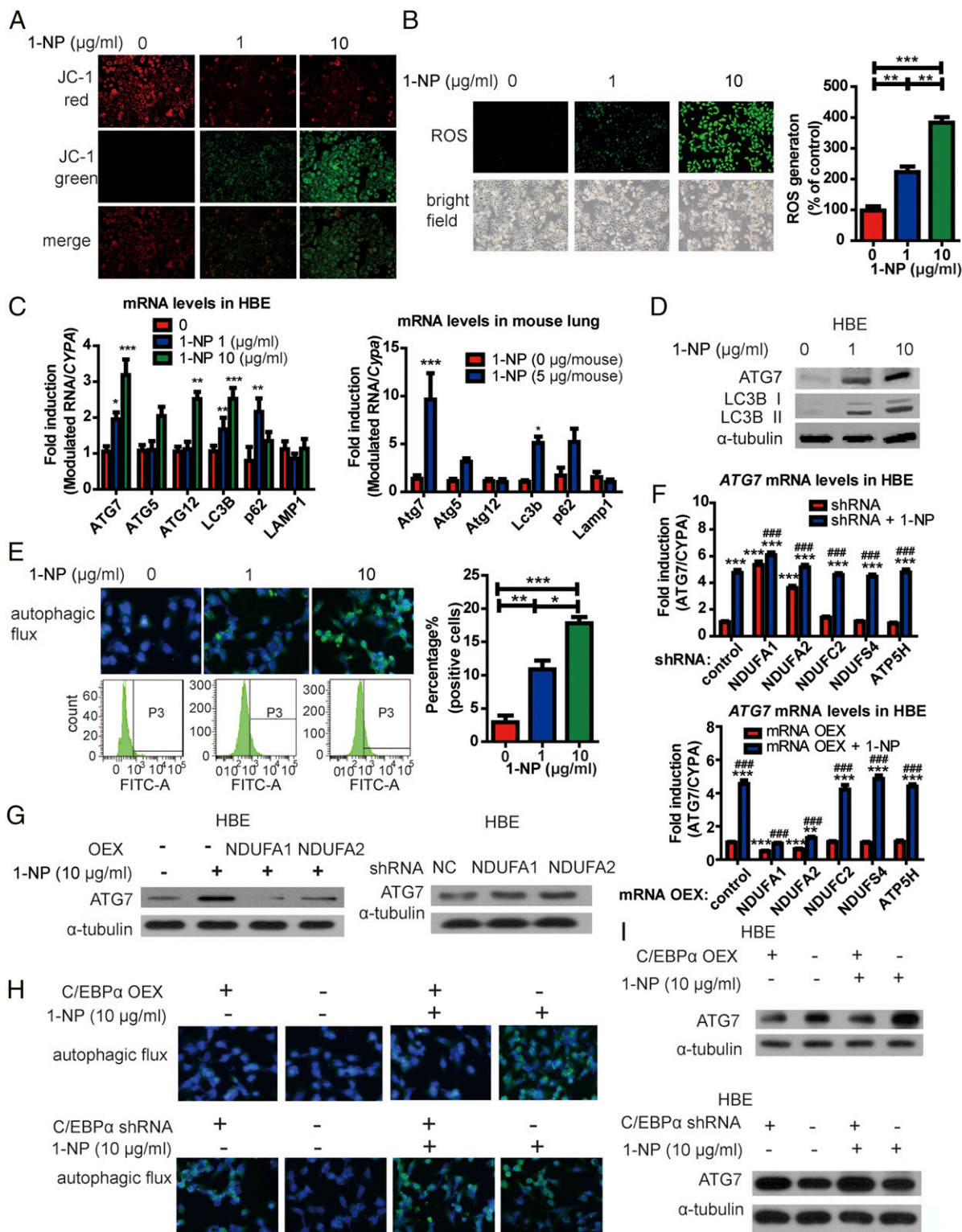


Fig. 2. 1-NP increases mitochondrial dysfunction and autophagy in HBE cells. (A) JC-1 staining suggested collapse of MMP in 1-NP-treated HBE cells. (B) ROS generation increased in 1-NP-treated HBE cells ($n = 3$). (C) mRNA expression levels of autophagy-associated genes in 1-NP-treated HBE cells and mouse lung tissue ($n = 6$). (D) Representative Western blot shows expression of ATG7 and LC3B in 1-NP-treated HBE cells. (E) Cyto-ID-labeled autophagosome puncta and was measured by flow cytometry ($n = 3$). (F) Inhibition or overexpression (OEX) of *NDUFA1* and *NDUFA2* increases or decreases the mRNA ($n = 6$) and (G) protein expression levels of ATG7 in HBE cells. (H) *C/EBPα* inhibits autophagic flux in HBE cells. (I) *C/EBPα* inhibits 1-NP-increased ATG7 expression. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with the control. ### $P < 0.001$ compared with the control within each group.

To assess upstream triggers of the autophagic flux, shRNA and lentiviral overexpression of *C/EBP α* or five mitochondria-related genes was introduced into HBE cells with or without 1-NP exposure. Repression of *NDUFA1* and *NDUFA2*, but not *NDUFC2*, *NDUFS4*, or *ATP5H*, enhanced mRNA and protein

expression levels of ATG7. Overexpression of *NDUFA1* and *NDUFA2* restored the levels of ATG7 to indistinguishable from controls following 1-NP exposure (Fig. 2 F and G). *C/EBP α* overexpression inhibited 1-NP-induced autophagic flux and ATG7 protein expression. Furthermore, inhibition of *C/EBP α*

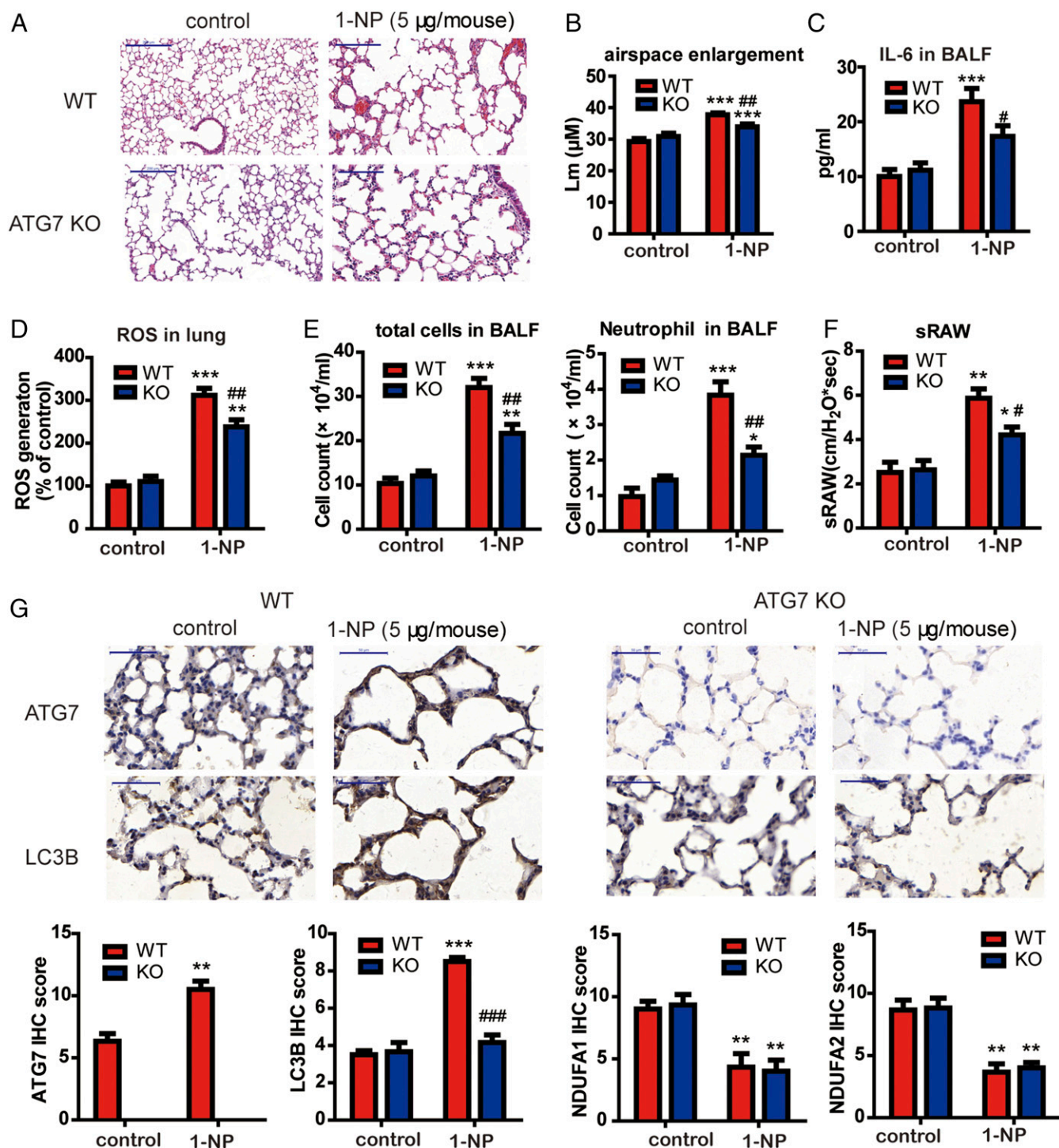


Fig. 3. Suppression of autophagy attenuates pulmonary disorders in mice. (A) Representative images of H&E staining in mouse lung tissues (100 \times). (Scale bar: 200 μ m.) (B) Histological score of mouse lung tissue ($n = 18$). (C) The IL-6 level in BALF ($n = 6$). (D) ROS generation in lung tissue. (E) The number of inflammatory cells in BALF ($n = 6$). (F) Airway responsiveness of mice ($n = 6$). (G) Representative images of IHC staining in mouse lung tissues and the histological scores (400 \times). (Scale bar: 50 μ m.) ($n = 6$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with the WT control. # $P < 0.05$ and ### $P < 0.001$, compared with the WT within each group.

increased Cyto-ID–labeled puncta and ATG7 expression in HBE cells (Fig. 2 *H* and *I*). Thus, the results suggest that 1-NP triggers autophagy in a C/EBP α /mitochondria/ATG7 pathway both in vivo and in vitro; however, the role that autophagy plays in the pathogenesis of 1-NP exposure is still unclear.

ATG7-Deficient Mice Are More Resistant to Experimental COPD. The findings presented above suggest a potential role for autophagy in 1-NP–induced pulmonary disorders. To address this hypothesis, wild-type (WT) and ATG7-knockout (KO) mice were treated with control medium (PBS) or a single dose of 1-NP, and the ensuing pulmonary functions were evaluated.

1-NP–exposed WT mice developed a significant airspace enlargement; in contrast, ATG7 KO mice were partially resistant to the 1-NP–induced emphysema and showed a diminished distal airspace size compared with 1-NP–exposed WT mice (Fig. 3 *A* and *B*). 1-NP exposure induced severe alveolitis in lung tissue of WT mice and moderate-to-severe alveolitis in ATG7-KO mice (SI Appendix, Fig. S9). Generation of ROS in lung tissue and secretory IL-6 levels [a cytokine associated with COPD exacerbations and decline in lung function (24)] in bronchoalveolar lavage fluid (BALF) were consistent with the severity of the emphysema (Fig. 3 *C* and *D*). 1-NP exposure resulted in marked accumulation of total cell number and neutrophils in the BALF

of both WT and ATG7-KO mouse, while a smaller number of inflammatory cells were observed in BALF of ATG7-KO mice compared with WT mice (Fig. 3*E*). Specific airway resistance (sRAW) [indices to identify acute bronchial response in COPD (25)] was measured in conscious mice, and hyper airway responsiveness was measured after exposure to 1-NP. After 1-NP exposure, sRAW in ATG7-KO mice was significantly lower than in WT mice (Fig. 3*F*). Expression of ATG7, LC3B, NDUFA1, and NDUFA2 was evaluated in lung tissue by immunohistochemistry (IHC) (Fig. 3*G* and SI Appendix, Fig. S10). ATG7 expression was elevated in 1-NP–exposed WT lung tissue and completely absent in ATG7-KO mice. Compared with WT mouse, LC3B expression increased in 1-NP–treated WT mice, but was unaltered in ATG7-KO mice. Lung NDUFA1 and NDUFA2 protein expression was significantly reduced in 1-NP–treated WT and ATG7-KO mice, compared with control WT mice. Taken together, our findings support the notion that depletion of autophagy could block pulmonary lesions induced by 1-NP in vivo.

Metabolomics Analysis Shows Modulated Metabolites of DEP-Treated HBE Cells. Metabolomics analysis was employed to identify a molecule that can potentially rescue mitochondrial complex I dysfunction and autophagy. We first assessed key metabolic

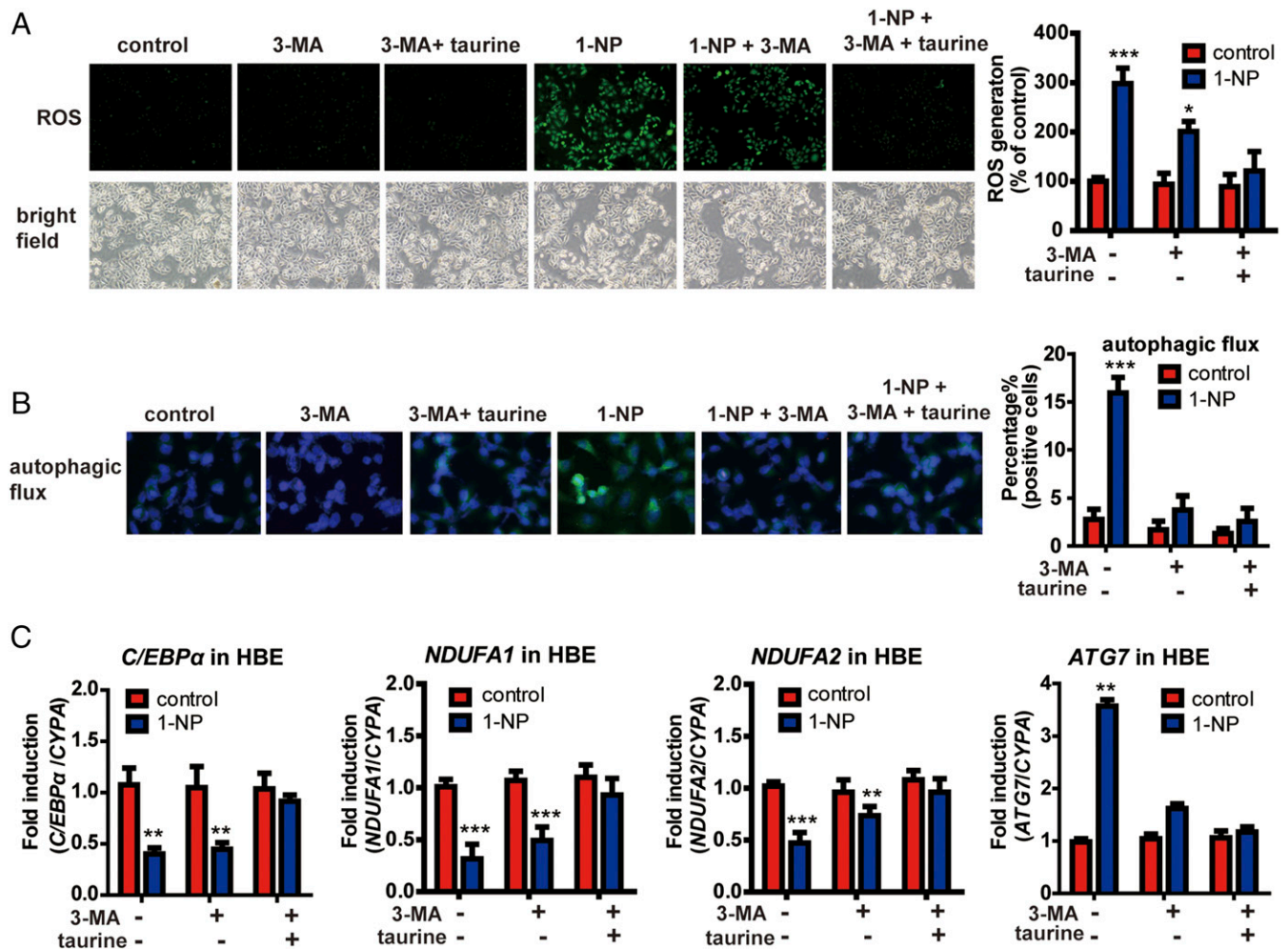


Fig. 4. Taurine and 3-MA rescue 1-NP–induced damage in vitro in HBE cells. (A) 3-MA and taurine decrease the generation of ROS in 1-NP–treated HBE cells ($n = 3$). (B) 3-MA and taurine inhibit autophagy in 1-NP–treated HBE cells ($n = 3$). (C) mRNA expression of *C/EBP α* , *NDUFA1*, *NDUFA2*, and *ATG7* was rescued by 3-MA and taurine ($n = 6$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, compared with the control.

differences between healthy and COPD patients and identified a total of 11 significantly modulated metabolites in the serum of COPD patients compared with serum of healthy subjects with a cutoff of FC > 1.5 and P value < 0.05 (SI Appendix, Table S7).

In the cellular lysates of untreated and DEP-treated HBE cells, three significantly regulated small molecules were identified after Bonferroni correction (P cutoff < 0.0001) (SI Appendix, Table S8). Notably, in HBE cells, deregulation of 3-methyladenine (3-MA) [a well-known inhibitor of autophagy (26)] and taurine [a

regulator of mitochondrial oxidative stress (27)] was concentration-dependent, and these two metabolites were also significantly decreased in COPD patient serum samples. Therefore, 3-MA and taurine were identified as candidates to rescue 1-NP-induced cellular damages.

Taurine Rescues Mitochondrial Dysfunction in Vitro. In the metabolomics analysis, 3-MA and taurine were shown to ameliorate mitochondrial dysfunction induced by 1-NP. The 3-MA (5 μ M) was administered to HBE cells before 1-NP exposure

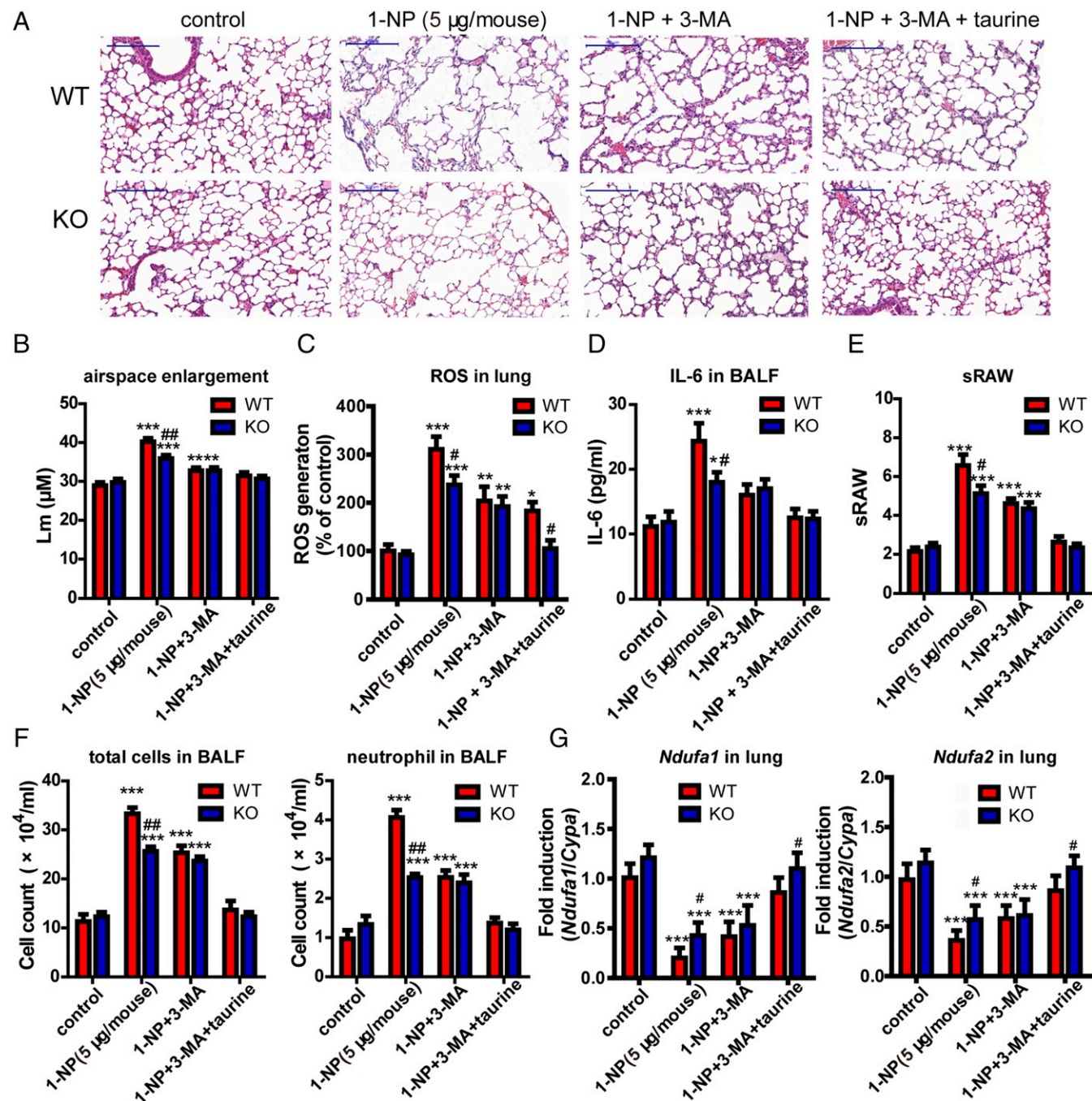


Fig. 5. Taurine and 3-MA ameliorate experimental COPD in mice. (A) Representative images of H&E staining in mouse lung tissues (100 \times). (Scale bar: 200 μ m.) (B) Histological scores of mouse lung tissues ($n = 18$). (C) ROS generation in lung tissues ($n = 6$). (D) IL-6 levels in BALF ($n = 6$). (E) Airway responsiveness of mice ($n = 6$). (F) Number of inflammatory cells in BALF ($n = 6$). (G) mRNA expression levels of *Ndufa1* and *Ndufa2* were rescued by 3-MA and taurine in 1-NP-treated mouse lung tissues ($n = 6$). * P < 0.05 and *** P < 0.001 compared with the WT control. # P < 0.05 and ## P < 0.01, compared with the WT within each group.

and partially ameliorated the collapse of the mitochondrial membrane potential (MMP). Furthermore, taurine (25 mM) coupled with 3-MA completely restored the MMP to control levels (*SI Appendix, Fig. S11A*). Consistent trends were observed in ROS generation in HBE cells with pretreatment of 3-MA or 3-MA coupled with taurine (Fig. 4A). The autophagosomes labeled by Cyto-ID in HBE cells were completely absent upon pretreatment with 3-MA or 3-MA coupled with taurine (Fig. 4B). Moreover, mRNA expression levels of *C/EBP α* , *NDUFA1*, *NDUFA2*, and *ATG7* in HBE cells were fully restored to control levels by 3-MA and taurine supplementation (Fig. 4C). However, taurine, but not 3-MA, rescued *NDUFC2*, *NDUFS4*, and *ATP5H* expression (*SI Appendix, Fig. S11B*). 3-MA shows the capacity to inhibit autophagy, but not to rescue the upstream gene expression levels. Taurine shows a broader spectrum than 3-MA to rescue the 1-NP-disturbed molecular pathway in vitro.

Taurine Ameliorates Experimental COPD Induced by PM. To further characterize the protective effects of taurine in vivo, WT and ATG7-KO mice were pretreated with 3-MA or 3-MA coupled with taurine before a single-dose administration of 1-NP. Cotreatment with 3-MA and taurine attenuated distal emphysema (Fig. 5A and B) and alveolitis (*SI Appendix, Fig. S12*) in both WT and ATG7-KO mouse lung tissue. Furthermore, the ROS generation in murine lung tissue and IL-6 levels in BALF were consistent and corroborated the histological alterations (Fig. 5C and D). Airway responsiveness recovered to control levels upon cotreatment with 1-NP, 3-MA, and taurine (Fig. 5E). Likewise, the accumulation of inflammatory cells and neutrophils in BALF was significantly reduced to normal levels after 3-MA and taurine rescue (Fig. 5F). Expression of mitochondria-related genes was completely rescued by cotreatment with taurine and 3-MA (Fig. 5G and *SI Appendix, Fig. S13*).

To ascertain the effects of taurine and 3-MA neutralization on DEP- or PM-induced emphysema, WT and ATG7 KO mice were challenged with DEP or PM. Representative images of lung tissues following DEP exposure are shown in *SI Appendix, Fig. S14A*, where the airspace enlargement can be seen to be reduced by taurine and 3-MA treatment. The alterations in sRAW, total cell number, and neutrophil number in BALF were consistent with the

trend for mean linear intercept (*SI Appendix, Fig. S14 B–D*). Likewise, taurine coupled with 3-MA treatment neutralized PM-induced lesions in mouse lungs (*SI Appendix, Fig. S14 E–H*). Results mentioned above verified the potential application of taurine to protect against PM-induced pulmonary disorders.

Key Molecular Pathways Involved in PM-Induced COPD. Our results suggest that PM induces pulmonary damage through mitochondria dysfunction by suppressing *C/EBP α* and NADH dehydrogenase-associated gene expression. In turn, enhanced ATG-7 expression increases oxidative stress and autophagy, leading to reduced murine pulmonary function. Using metabolomics analysis, we found that supplementation with the small molecular metabolite taurine, coupled with 3-MA, might rescue PM-induced lung dysfunction (Fig. 6), highlighting a potential therapy for PM-induced COPD.

Discussion

We report on the association between PM exposure and increased risk of COPD and establish that PM-induced COPD results from the switching off of the mitochondrial complex I function. Treatment with taurine and 3-MA efficiently rescues pulmonary function from the PM-induced alterations.

Epidemiological studies have indicated that exposure to DEP, a significant contributor to ambient PM, is associated with health impairments, including COPD (28, 29). An in vitro study suggested that a hallmark of airway diseases including emphysema and COPD, matrix metalloproteinase-1, has been up-regulated by DEP in HBE cells (30). However, the underlying mechanisms of PM-induced COPD have yet to be delineated. Our mRNA microarray data suggest a strong association between mitochondria complex dysfunction and DEP exposure. 1-NP, a probable human carcinogen (group 2A) (31), was identified as playing a prominent role in the pathogenicity of experimental COPD.

Mitochondrial function is associated with COPD pathogenesis. Damaged mitochondrial structures, such as depletion of cristae, increased branching, and swelling, were previously observed in airway epithelial cells of patients with advanced COPD (32). Nonetheless, little is known about the functional state of mitochondrial complexes in response to environmental stress in patients with COPD. A previous study reported that short-term cigarette smoke exposure in mice resulted in a higher expression

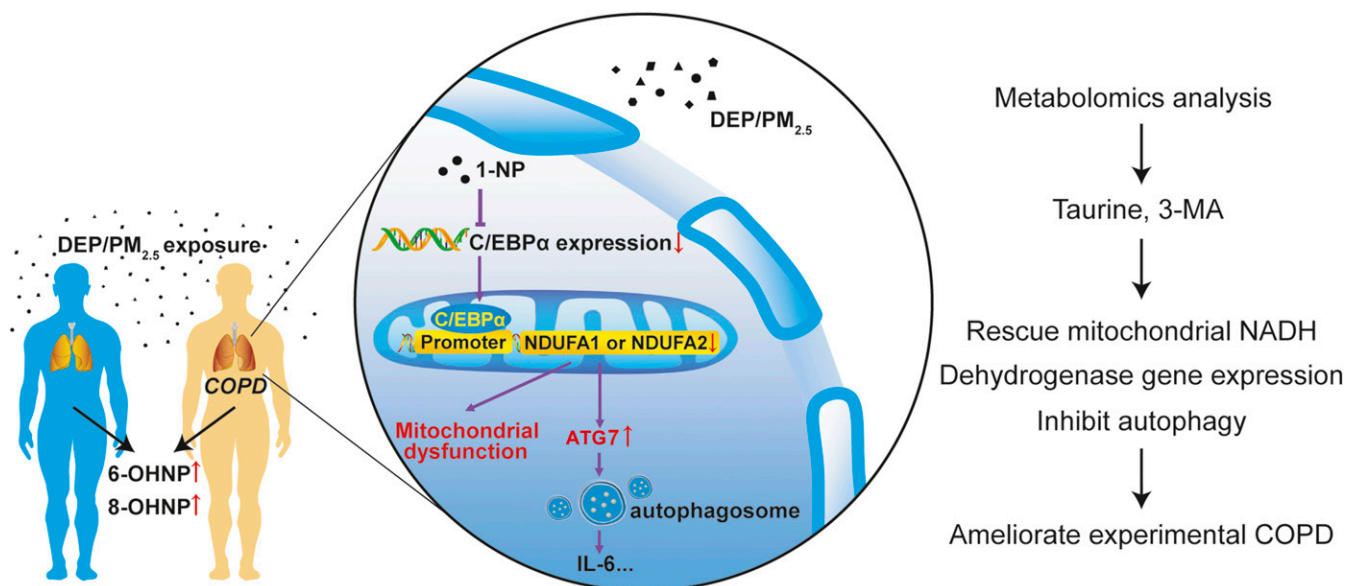


Fig. 6. The key molecular pathway involved in PM-induced damage.

of complexes II, III, IV, and V, which might be viewed as a compensatory response to reduced pyruvate supplement (33). Wiegman et al. (34) suggested that increased ozone-induced oxidative stress exacerbated the COPD-like phenotype in mouse lung tissue. Consistent with these observations, our data on compromised NADH-mediated respiration corroborated earlier observations of mitochondrial damage in human subjects with COPD (32).

Our results suggest that C/EBP α transcription drives mitochondrial NADH dehydrogenase dysfunction. C/EBP α is a basic leucine zipper transcription factor that controls cell proliferation, differentiation, and proinflammation. Depletion of C/EBP α has been associated with a reduced number and size of mitochondria, contributing to impaired mitochondrial biogenesis (35). In addition, C/EBP α plays a role as a tumor suppressor in lung tissue. Reduced expression of C/EBP α promoted the proliferation of lung cancer (36, 37). In contrast, enhanced expression of C/EBP α effectively inhibited growth of human lung cancer cells both in vitro and in vivo (38).

We hypothesize that, as a consequence of compromised complex I function, autophagy increases in pulmonary cells, leading to inflammation and emphysematous destruction in experimental COPD. Consistent with our observations, a recent study reported that autophagy is critical in mediating inflammation and mucus hyper-production in epithelia via NF- κ B and AP-1 pathways following exposure to inhalable ambient particles (18). Our results support the notion that autophagy plays a deleterious role in PM-induced lung damage, given that the autophagy inhibitor, 3-MA, partially ameliorated the PM-induced pulmonary lesions by inhibiting ATG-7-mediated autophagic pathway. A recent study also suggested that 3-MA might rescue murine pulmonary cell death in response to nanoscale particle exposure (39).

Finally, we highlight the efficacy of therapeutically targeting mitochondrial complex I in COPD. Our studies suggest that antioxidant-rich diets or chemicals might be beneficial in mitigating the effects of air pollution by counteracting the associated oxidative stress (6, 40). It has been reported that taurine is an important regulator of oxidative stress, and decreased taurine content has been shown to trigger a decline in respiratory chain complexes I and III activity (41). Taurine was used to prevent the ethanol-induced decrease in C/EBP α expression level in rat liver due to its antioxidant capacity (42). Bai et al. (43) also recently showed that taurine rescued cells from As₂O₃ damage by inhibiting autophagy and oxidative stress in livers of rat offspring. In the present study, treatment of taurine coupled with 3-MA completely rescued mitochondrial NADH dehydrogenase function, affording airway epithelia protection from PM-induced injuries.

In summary, we show an association between the major toxic components of PM with increased COPD risk. The molecular pathway C/EBP α /mitochondria/autophagy is shown here to mediate pulmonary responses to PM. Suppression of autophagy attenuated PM-induced pulmonary disorders; furthermore, treatment with taurine restored mitochondrial function both in vivo and in vitro. These findings have significant translational implications regarding the understanding of COPD-like lesions induced by

PM. The exquisite role of mitochondria in this signaling cascade suggests that mitochondrial complex I might be an effective target for preventive intervention of ambient air-pollution-induced COPD.

Materials and Methods

Complete details of all reagents and procedures used in this study are provided in *SI Appendix, SI Materials and Methods*.

Subject Characterization. We recruited 600 healthy volunteers and 120 COPD patients for the present study. The Institutional Review Board in the School of Public Health at Southeast University approved the study protocol. Written consent forms were obtained from all subjects. Details are provided in *SI Appendix, SI Materials and Methods*.

Cell Culture. The HBE cell line (American Type Culture Collection) was maintained in DMEM at 37 °C in 5% CO₂. The culture medium was supplemented with 10% (vol/vol) FBS.

Animal Experiments. Animals were treated humanely, and all experimental protocols were approved by the Committee on Animal Use and Care of Southeast University (approval no. SEU2015030031). All of the methods in the present study were performed according to approved guidelines. Five mice were housed in each polycarbonate cage on corn cob bedding with ad libitum access to food and water. Light cycles were set on a 12 h/12 h light/dark cycle, and room temperature was set to 22.5 °C.

Details are provided in *SI Appendix, SI Materials and Methods*.

Mitochondria Functional Analysis and ROS Generation. HBE cells and lung tissue from mice were collected after treatment. The mitochondrial membrane potential in HBE cells and levels of ROS in HBE cell lysates and lung tissues were measured by flow cytometry as previously described (20).

Data Analysis. All statistical tests were two-sided, and the significance was set at $P < 0.05$. Significant differences were determined by one-way ANOVA, followed by Tukey's multiple comparison tests. The Kruskal–Wallis test was used to analyze the ranked data of pulmonary lesion scores. Paired t tests were used to compare the indicators of the panel study. The linear correlation was determined by Pearson correlation analysis in the panel study. The $2^{-\Delta\Delta Ct}$ method was used to analyze the qRT-PCR results in all experiments. Statistical analysis was performed by SPSS 12.0.

Data Deposition. The microarray datasets reported in this paper have been deposited in the Gene Expression Omnibus repository (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE82335>).

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