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ORIGINAL RESEARCH

miRNA-486-5p Promotes COPD Progression by Targeting HAT1 to Regulate the TLR4-Triggered Inflammatory Response of Alveolar Macrophages

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Purpose: The aim of this study was to investigate the role of *miRNA-486-5p* in chronic obstructive pulmonary disease (COPD) progression and the underlying molecular mechanisms.

Materials and Methods: Aberrant miRNA expression profiles between smokers and nonsmokers, and those between COPD patients and normal subjects were analyzed using microarray datasets and reverse-transcriptase quantitative polymerase chain reaction (qPCR). Enzyme-linked immunosorbent assay was used to determine the levels of inflammatory cytokines in cell supernatants. Expression levels of inflammatory cytokines, HAT1, TLR4, and *miR-486-5p*, were determined using qPCR or Western blotting. Luciferase reporter assays and fluorescence in situ hybridization were used to confirm the regulatory interaction between *miR-486-6p* and *HAT1*.

Results: *miR-486-5p* was significantly upregulated in the COPD and smoker groups compared to the control group, as demonstrated using bioinformatics analysis and validated using qPCR assay of alveolar macrophages and peripheral monocytes. Moreover, miR-486-5p expression was significantly correlated with the expression of IL-6, IL-8, TNF-α, and IFN-γ. Luciferase reporter assays confirmed that *miR-486-5p* directly targeted *HAT1*, and cellular localization showed that *miR-486-5p* and HAT1 were highly expressed in the cytoplasm. *miR-486-5p* overexpression led to a significant upregulation of TLR4 and a significant downregulation of HAT1. Inversely, *miR-486-5p* inhibition led to a significant downregulation of TLR4 and a significant upregulation of HAT1. *HAT1* knockdown using siRNA significantly upregulated the expression of TLR4, IL-6, IL-8, TNF-α, and IFN-γ.

Conclusion: *miR-486-5p* was differentially expressed in the alveolar macrophages of COPD patients. *miR-486-5p* overexpression may enhance the *TLR4*-triggered inflammatory response in COPD patients by targeting *HAT1*.

Keywords: smoking, chronic obstructive pulmonary disease, miR-486-5p, toll-like receptor 4, histone acetyltransferase 1

Introduction

Subdermal Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disorder associated with aging and smoking, and it has been a major public health problem owing to its high prevalence, morbidity, and mortality.[1] Environment (especially smoking) and genetics are the two major factors responsible for COPD etiology. In Western countries, more than 90% of the COPD cases are caused by chronic smoking.[2,3] The immunopathology of COPD is associated with innate and adaptive

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inflammatory immune responses to chronic smoking.[4] The central feature of COPD is the infiltration of alveolar macrophages, neutrophils, and other inflammatory cells into the lung parenchyma and peripheral airways. Moreover, the predominant inflammatory cell types vary with COPD severity.[4,5] Therefore, a better knowledge of inflammatory responses and the corresponding intracellular signaling pathways is essential for COPD drug development and clinical treatment.

Toll-like receptors (TLRs) are a class of evolutionarily conserved receptors of the innate immune system and are known to play crucial roles in host defense by triggering innate and adaptive immunity.[6,7] TLRs contribute to the pathogenesis of inflammation by inducing inflammatory cytokines and other endogenous molecules in response to pathogenic microbial infection.[8,9] TLR4 is a well-studied TLR family member, but its role in COPD immunopathology is not completely clear. Reportedly, TLR4 promotes airway neutrophilia in COPD,[10] and tagging single nucleotide polymorphisms in TLR4 are associated with high numbers of sputum inflammatory cells and lung function decline.[11] In a previous study, higher expression levels of TLR4 were observed in the bronchial mucosa of patients with severe COPD compared to those in the mucosa of healthy individuals, and TLR4 overexpression was positively correlated with CD4+/CD8+ cell infiltration and airflow obstruction.[12] These results suggest that TLR4 plays an important role in COPD pathogenesis and progression.

MicroRNAs (miRNAs) are 21–24 nt long non-coding RNAs that regulate gene expression at the post-transcriptional level. Recent research has demonstrated the crucial regulatory roles of miRNAs in many diseases, including COPD.[13,14] The upregulation of *miR-34a* has been shown to promote cellular senescence in small airway fibroblasts of COPD patients.[15] Moreover, overexpression of *miRNA-125a/b* in COPD results in heightened airway inflammation by inducing A20, which mediates NF-κB activation, while inhibition of *miRNA-125a/b* decreases the induction of inflammatory cytokines.[16] Here, we provide evidence that *miR-486-5p* mediates the TLR4-triggered inflammatory response of alveolar macrophages in COPD. We found that *miR-486-5p* was upregulated in alveolar macrophages and peripheral monocytes of COPD patients and smokers compared to that in the alveolar macrophages and peripheral monocytes of controls, and its expression was positively correlated with the expression levels of IL-6, IL-8, TNF-α, and IFN-γ. In addition, *miR-486-5p* regulated the TLR4-triggered inflammatory response by targeting histone acetyltransferase 1 (HAT1). Thus, our findings provide novel targets and a theoretical basis to further investigate the pathogenesis of COPD and may eventually contribute to the therapeutic management of COPD.

Materials and Methods

CSE

For the preparation of CSE, three cigarettes without filters (0.9 mg flue gas nicotine, 11 mg coke, and 14 mg flue gas carbon monoxide) were collected using a negative pressure suction device and poured into a flask with 3 mL phosphate-buffered saline. After shaking well, the suspension was filtered using a 0.22 μm membrane filter to remove the bacteria. To ensure a similar concentration of CSE in all the preparations, the absorbance value for each preparation was determined at the optimal absorption wavelength (270–280 nm). The CSE concentrate was diluted using 10% fetal bovine serum containing Roswell Park Memorial Institute-1640 culture medium to obtain 2%, 5%, and 10% CSE. Notably, CSE was prepared 30 min before use to ensure the effectiveness of the CSE ingredients.

Cell Culture and Treatment

The rat pulmonary alveolar macrophage cell line NR8383 was purchased from Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd. and cultured in F12K medium supplemented with 15% fetal bovine serum at 37°C in a humidified mixture of air (95%) and CO2 (5%). Cells were plated in 6-well plates at a density of 1 × 106 cells per well and cultured in serum-free F12K medium overnight. Cells were treated with CSE for 24 and 48 h.

qPCR

Total RNA extraction was performed using Trizol reagent according to the manufacturer's instructions. After determining the RNA concentration and quality, complementary DNA (cDNA) was synthesized using a First Strand cDNA Synthesis Kit (Sangon Biotech, China). qPCR was performed to validate gene expression using 2× SYBR Green PCR Master Mix (Sangon Biotech, China) on an Mx3000P QPCR System (Stratagene, USA) with the following thermal cycling conditions: 95°C for 3 min, followed by 40 cycles at 95°C for 12 s and 62°C for 40 s. The specific primer pairs used are shown in Supplemental Table 1.

Western Blotting

Total protein extraction was performed using the M-PER Mammalian Protein Extraction Reagent according to manufacturer's instructions. Following this, proteins were separated using a 10% sodium dodecyl sulfate-polyacrylamide gel. The proteins were transferred onto polyvinylidene difluoride membranes (Millipore) using a semi-dry cell at 30 mA for 60 min, and then, 1×Ponceau S solution was used to dye and mark the protein marker spots. Blocking buffer (1×Tris buffered saline (TBS), 0.1% Tween-20 (TBST), and 5% w/v non-fat dry milk) was used to block nonspecific binding for 2 h at 37°C. After washing three times with 1×TBST, the membranes were incubated with anti-HAT1 (1:1000, SAB) and anti-TLR4 (1:1000, SAB) primary antibodies at 4°C overnight. The membrane was washed with 1×TBST five times, followed by incubation with HRP-secondary antibodies (1:2000, Jackson) for 2 h at 37°C. After washing five times with 1×TBST, the membrane was exposed to SuperSignal West Pico Chemiluminescent Substrates, and gray scanning was performed using a Gel-Pro Analyzer.

Luciferase Reporter Assays

NR8383 cells were seeded onto 96-well plates for 15–18 h of culture. Next, 5 pmol *miR-486-5p* mimic/negative control (NC) and 0.16 μg HAT1 3′ UTR/HAT1 3′ UTR-muta (Sangon Biotech, China) were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The medium was replaced with fresh medium after transfection for 6 h. Luciferase activity of the reporter vectors was determined after 48 h, using the Promega Dual-Luciferase system according to the manufacturer's instructions.

ELISA

After CSE treatment, the cell supernatants were collected and analyzed using ELISA kits (Sangon Biotech, China) as per the manufacturer's instructions.

miRNA Mimics/Inhibitors and Small Interfering RNA

miR-486-5p mimics, inhibitors, and the corresponding NCs were synthesized by Sangon Biotech (Shanghai) Co., Ltd. and transfected into cells using Lipofectamine 2000 (Invitrogen) to over-express or inhibit *miR-486-5p* in cells. The specific sequences of the *miR-486-5p*-mimics, inhibitors, and HAT-1 specific siRNA are shown in Supplemental Table 2.

FISH

Cells were seeded in a confocal Petri dish and cultured for 24 h. After fixing with 4% paraformaldehyde, the cells were impregnated with 50%, 80%, and 98% (mass ratio) ethanol for 3 min before dehydration. The fixed cells were prehybridized in hybridization buffer (formamide, 50 mM Tris-HCl, 5 mol NaCl, and 0.05% sodium dodecyl sulfate) based on a previously described method. The 5′ oligonucleotide-labeled miR-486-5p probe sequence was 5′-Cy3-CUCGGGGCAGCUCAGUACAGGA, and the 5′ oligonucleotide-labeled HAT1 probe sequence was 5′-FAM-UUCUCCACCGCACUCUUAUAUU. Next, DAPI was added in the dark at 4°C for 5 min before washing with 4°C phosphate-buffered saline buffer. Cells were observed under a fluorescence microscope with a 360 nm excitation wavelength and a 460 nm emission wavelength filter.

Clinical Sample Collection

The bronchoalveolar lavage fluid was collected from 14 healthy individuals and 36 COPD patients. Next, the PAM were isolated. Briefly, the bronchoalveolar lavage fluid was centrifuged at 4°C at 1500 rpm for 10 min. After discarding the supernatant, the cells were washed twice with Hanks' solution. The cells were cultured in serum-free Roswell Park Memorial Institute medium-1640 at 37°C in an incubator with 5% CO2 and 100% humidity for 2–3 h. Non-adherent cells were removed such that the cells adhering to the culture dish wall were PAM. Peripheral blood was collected from 128 participants (nonsmokers: n=33; smokers: n=42; COPD I–II: n=30; COPD III–IV: n=23). Monocytes were isolated from peripheral blood, and monocyte-derived macrophages were induced. All participants were recruited between January 2019–1 solstice and June 30, 2019, at the respiratory department and physical examination center at the Affiliated Huai'an Hospital of Xuzhou Medical University. Written informed consent was obtained from the participants before sample collection. This study was conducted under the approval of the Ethics Committee of the Affiliated Huai'an Hospital of Xuzhou Medical University.

Microarray and Data Analysis

The microarray datasets GSE38974 and GSE53519 were downloaded from the GEO database. The miRNA

expression data of lung tissue from 19 subjects with COPD and eight normal smokers without COPD in GSE38974 and the miRNA expression data of small airway epithelium from nine nonsmokers and ten smokers in GSE53519 were used in this study. Differential expression analysis was performed using the Bayesian method in the Limma package. The mRNA targets of miRNAs were predicted using miRTarBase[41] (http://mirtarbase.mbc.nctu.edu.tw/php/index.php), TargetScan[42] (http://www.targetscan.org/vert_72/), miRWalk 2.0[43] (http://mirwalk.umm.uni heidelberg. de/), and miRPathDB[44] (https://mpd.bioinf.uni-sb.de/overview.html). The overlapped mRNAs were selected using Venny analysis.

Statistical Analysis

Data analyses were conducted using SPSS 19.0 and GraphPad Prism 7. Results are expressed as the mean ± standard deviation (SD). Student's *t*-test was used to compare data between the two groups. Variance analysis was performed to compare multiple groups. One-way analysis of variance was used for homogeneity of variances, and the Lsd-q test was selected for pairwise comparison between groups. The Welch method was used when the variances were uneven, and Dunnett's T3 method was used for pairwise comparison among multiple groups. Differences were considered statistically significant at $P < 0.05$.

Results

Elevated Levels of Inflammatory Cytokines with Cigarette Smoke (CSE) Extract Treatment

The mRNA expression levels of IL-6/-8, IFN-γ, and TNF-α were significantly higher in CSE-treated NR8383 cells compared to those in the controls ($P < 0.05$, Figure 1A). Consistently, enzyme-linked immunosorbent assay (ELISA) revealed increased concentrations of IL-6/-8, IFN-γ, and TNF-α in CSE-treated NR8383 cells compared to those in the controls ($P < 0.05$, Figure 1B).

Upregulation of miR-486-5p in COPD

Based on the GSE38974 dataset, 17 differentially expressed miRNAs were screened between the normal and COPD groups, and miR-486-5p was found to be highly expressed in COPD samples compared to normal samples (Figure 2A). In addition, based on the data from GSE53519, 45 differentially expressed miRNAs were screened between smoker and nonsmoker groups. Notably, *miR-486-5p* was significantly upregulated in the small airway epithelium of smokers compared to that in the controls (Figure 2B). *miR-486-5p* expression was evaluated using qPCR in alveolar macrophages (PAM) and peripheral monocytes to confirm bioinformatic data. Consistently, higher levels of *miR-486-5p* were observed in the PAM and peripheral monocytes of COPD patients than those in the controls. Moreover, *miR-486-5p* was highly expressed in the peripheral monocytes of smokers (Figure 2C and D).

CSE Results in Elevated Levels of Inflammatory Cytokines via miR-486-5p

To determine the association between *miR-486-5p* expression and inflammatory cytokine levels in cells treated with CSE, *miR-486-5p* mimic and inhibitor were used to induce its overexpression and inhibition, respectively. Notably, overexpression of *miR-486-5p* in NR8383 cells led to significant upregulation of IL-6/-8, IFN-γ, and TNF-α, which was enhanced further with CSE treatment (Figure 3A). Moreover, inhibition of *miR-486-5p* in NR8383 cells resulted in a significant downregulation of IL-6/-8, IFN-γ, and TNF-α (Figure 3B). These results indicate that *miR-486-5p* plays a role in the regulation of inflammatory responses in COPD.

Identification of HAT1 as a Target of miR-486-5p

The targeted mRNAs of *miR-486-5p* were predicted using miRTarBase, TargetScan, miRWalk, and miRPathDB. In total, 11 mRNAs were predicted, including *HAT1* (Figure 4A). Luciferase reporter assays and fluorescence in situ hybridization (FISH) assay were conducted to confirm the regulatory interaction between *miR-486-5p* and *HAT1*. As shown in Figure 4B, the luciferase activity of *HAT1*-WT was remarkably reduced ($P < 0.05$) after co-transfection with the miR-486-5p mimic, while the luciferase activity of the control and *HAT1*-MUT showed no significant difference (Figure 4B). This indicated that *miR-486-5p* could bind to the 3′-UTR region of *HAT1* mRNA to inhibit *HAT1* expression. Cellular localization showed that *miR-486-5p* and *HAT1* were highly expressed in the cytoplasm. *miR-486-5p* expression was elevated, while HAT1 expression was decreased in the *miR-486-5p* mimic and mimic+CSE groups compared to their expression in the control group. *miR-486-5p* inhibition resulted in significant upregulation of HAT1 in the *miR-486-5p* inhibitor and inhibitor+CSE groups compared to that in the control group (Figure 4C).

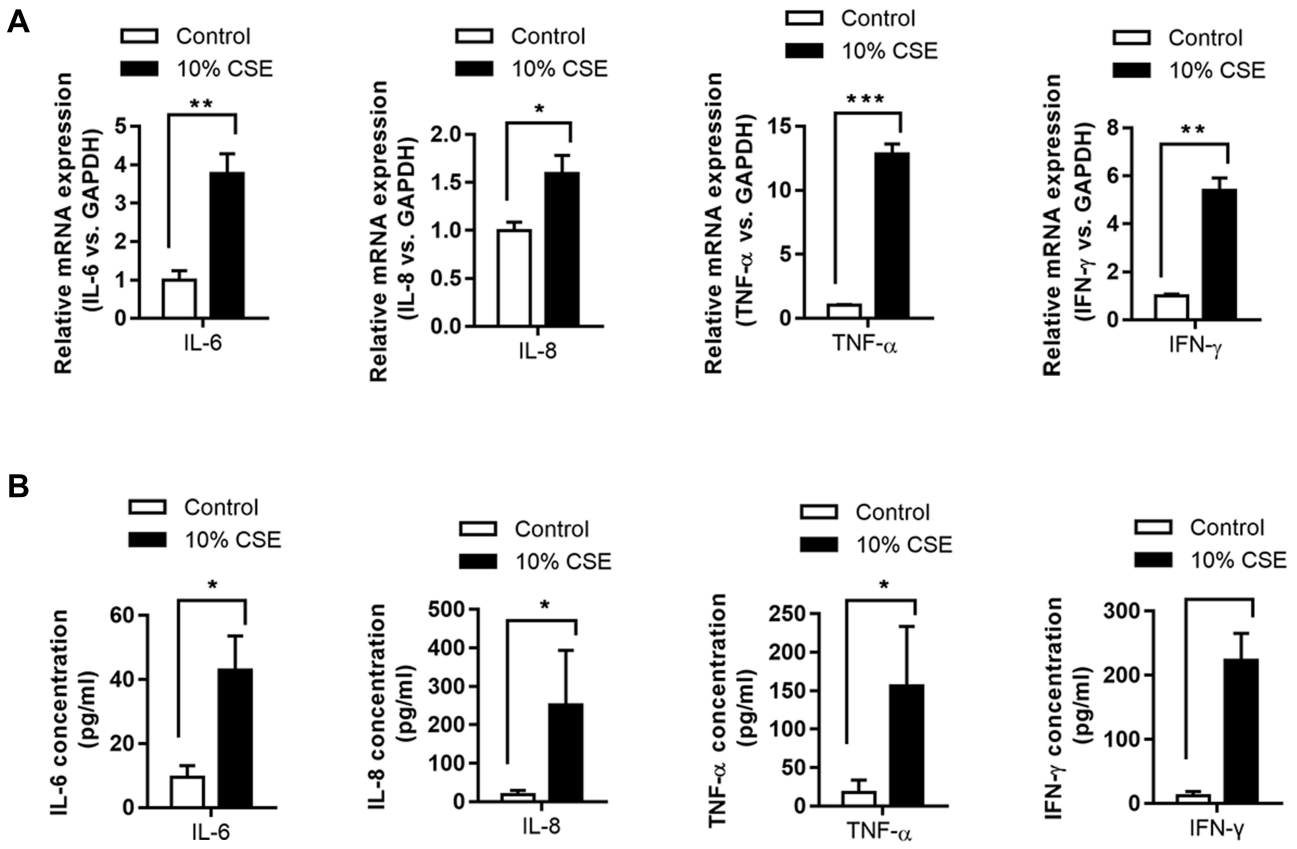

Figure 1 Levels of inflammatory cytokines in NR8383 cells treated with CSE. mRNA expression of *IL-6*, *IL-8*, *TNF-α*, and *IFN-γ* in 10% CSE-treated NR8383 cells was determined using qPCR (**A**). The levels of IL-6, IL-8, TNF-α, and IFN-γ in cell supernatants were determined using ELISA kits (**B**). *P < 0.05; **P < 0.01; ***P < 0.001.

miR-486-5p Regulates TLR4 Expression by Targeting HAT1

Studies have reported that TLR4 can trigger a complex inflammatory response.[17,18] Therefore, the interactions between *miR-486-5p* and *TLR4* were further explored. *miR-486-5p* and TLR4 were highly expressed in NR8383 cells treated with CSE, while *HAT1* expression decreased (Figure 5A). In addition, *miR-486-5p* overexpression in NR8383 cells led to an apparent increase in TLR4 expression and a significant downregulation of HAT1. Inversely, *miR-486-5p* inhibition in NR8383 cells resulted in decreased TLR4 expression and a significant upregulation of HAT1 (Figure 5B). We speculated that *miR-486-5p* regulates *TLR4* expression by targeting *HAT1* in COPD.

HAT1 Negatively Regulates TLR4 and Inflammatory Cytokine Expression

HAT1 siRNAs were used to inhibit HAT1 expression to confirm the regulatory interaction between *HAT1* and *TLR4*, and si-*HAT1*-697 showed a stronger knockdown effect (Figure 6A). Notably, *HAT1* inhibition by si-*HAT1*-697 resulted in a significant upregulation of TLR4 compared to si-NC and control. In contrast, TLR4 expression was significantly decreased by HAT1 overexpression (Figure 6B). Moreover, the expression levels of IL-6/-8, IFN-γ, and TNF-α were significantly increased by inhibiting HAT1 using si-HAT1-697 (Figure 6C). These results suggest that *miR-486-5p* promotes COPD progression by regulating the *TLR4*-triggered inflammatory response of alveolar macrophages by targeting *HAT1*.

Discussion

COPD is a multifactorial disorder characterized by nonreversible and progressive airflow obstruction with pulmonary dysfunction.[19] Although oxidative stress, immunity, inflammation, apoptosis, and other factors have been implicated in COPD, its exact pathogenesis is still obscure.[20,21] Smoking is one of the major causes of COPD. Investigation of the pathogenesis underlying smoking-related COPD is useful for early diagnosis and therapy. Studies have reported that some miRNAs can serve as important regulators in the molecular mechanisms of COPD.[22,23] The identification of

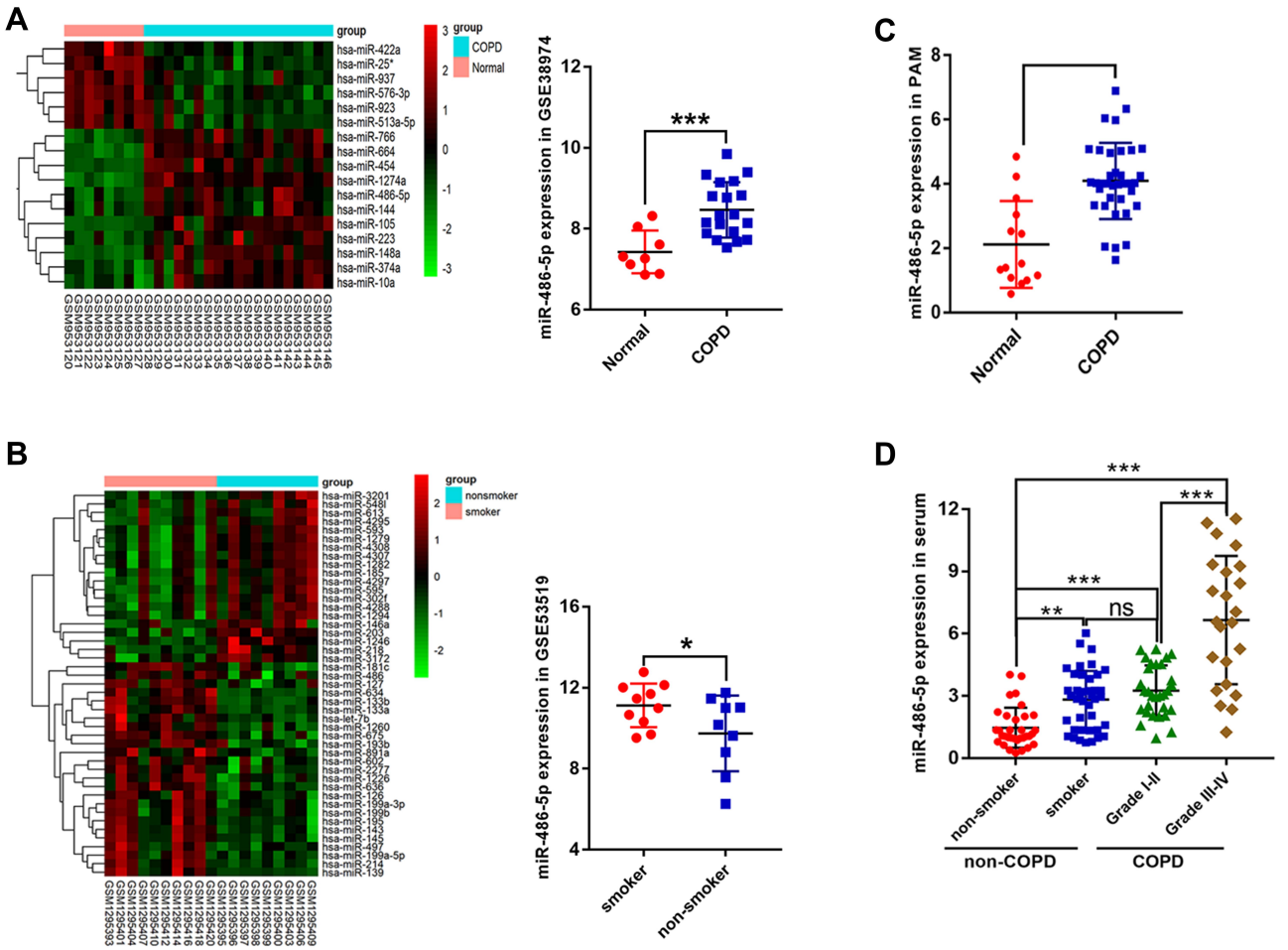

Figure 2 Screening and validation of differentially expressed miRNAs. Heat maps of differentially expressed miRNAs in COPD patients vs those in normal individuals, based on the GSE38974 dataset and *miR-486-5p* expression in the GSE38974 dataset (**A**). Heat maps of differentially expressed miRNAs in smokers vs those in nonsmokers, based on the GSE53519 dataset and *miR-486-5p* expression in the GSE53519 dataset (**B**). *miR-486-5p* expression in alveolar macrophages of COPD patients (n=36) and normal individuals (n=14), determined using qPCR (**C**). miR-486-5p expression in peripheral monocytes in smokers (n=42) vs that in the peripheral monocytes in nonsmokers (n=33) and in COPD I–II (n=30) vs COPD III–IV (n=23), determined using qPCR (**D**). *P < 0.05; **P < 0.01; ***P < 0.001.

aberrant miRNA expression in COPD could help to better understand the underlying mechanisms. It has been reported that miRNAs are abnormally expressed in COPD patients and smokers compared to those in the normal patients. For instance, Paschalaki et al demonstrated that downregulation of *miR-126* contributes to DNA damage in smokers and COPD patients.[24] Conickx et al suggested that *miR-218-5p* expression is decreased in COPD patients and smokers without airflow limitation, and its expression levels show a significant relationship with airway obstruction.[23] Here, miR-486-5p was aberrantly expressed in smokers and patients with COPD compared to that in nonsmokers without COPD, as demonstrated by bioinformatics analysis on a microarray dataset. This was consistent with *miR-486-5p* expression in the PAM and peripheral monocytes of COPD patients and smokers. This suggests that *miR-486-5p* is involved in COPD progression.

miR-486 is an intragenic miRNA located within Ankyrin 1.[25] Studies have reported that aberrant *miR-486-5p* expression can affect the occurrence and progression of many diseases.[26,27] Notably, the effect of *miR-486-5p* is controversial because it has been found to serve as a suppressor or oncogene.[28,29] In addition, we observed that the function of *miR-486-5p* had not been explored in COPD. Our results revealed that *miR-486-5p* expression was significantly correlated with the levels of IL-6/-8, IFN-γ, and TNF-α. Therefore, we suggest that *miR-486-5p* may play a role in mediating the inflammatory response. Chai et al reported that increased *miR-486-5p* levels repress the lipopolysaccharide-induced expression of inflammatory cytokines such as IL-1β, IL-6, and TNF-α.[30] TLRs play a role in inflammatory pathogenesis by inducing inflammatory cytokines and other endogenous molecules against pathogenic microbial infection.[8,9]

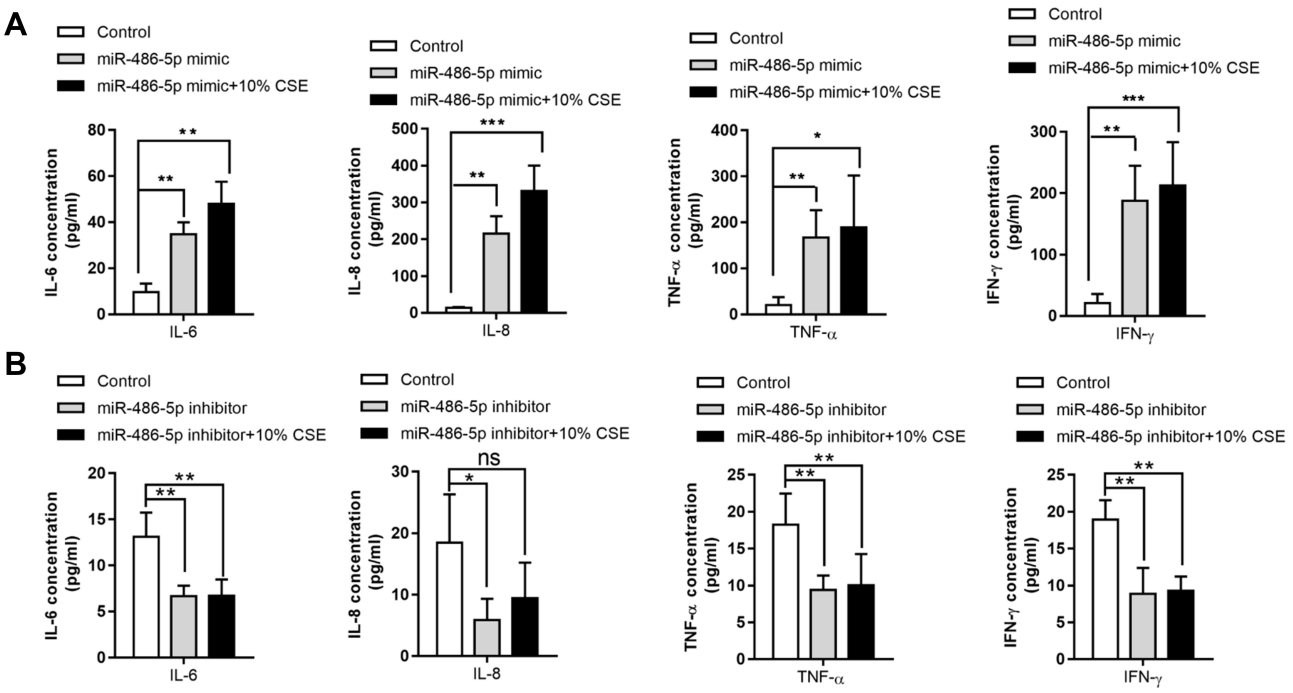

Figure 3 Association between inflammatory cytokines and *miR-486-5p* expression. IL-6, IL-8, TNF-α, and IFN-γ levels in cell supernatants were determined using ELISA kits. (**A**) IL-6, IL-8, TNF-α, and IFN-γ levels in cells after transfection with the *miR-486-5p* mimic. (**B**) IL-6, IL-8, TNF-α, and IFN-γ levels in cells after transfection with the *miR-486-5p* inhibitor. *P < 0.05; **P < 0.01; ***P < 0.001.

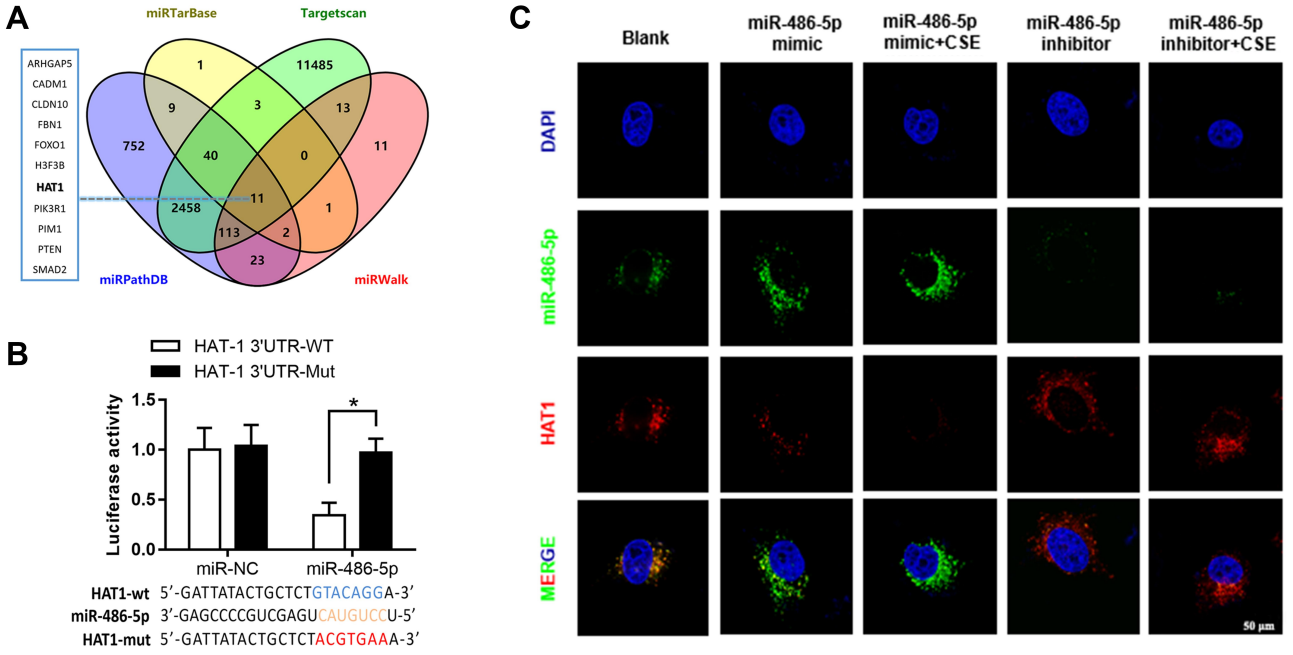

Figure 4 Regulatory interactions between *miR-486-5p* and *HAT1*. (**A**) Venn diagram of the predicted *miR-486-5p* targets in four databases. Results of the luciferase reporter assays (**B**) and fluorescence in situ hybridization (**C**) showing that *miR-486-5p* regulates the target *HAT1*. *P < 0.05.

Recently, miRNAs have been found to play crucial regulatory roles in TLR signaling. For example, Shen et al revealed that *miR-149-3p* affects the expression of IL-1β and TNF-α by regulating the TLR-4/NF-κB signaling pathways in COPD patients.[31] Lai et al suggested that *miR-92a* regulates the TLR4-triggered inflammatory response in macrophages via *JNK/c-Jun* signaling activated by mitogen-activated protein kinase 4.[32] However,

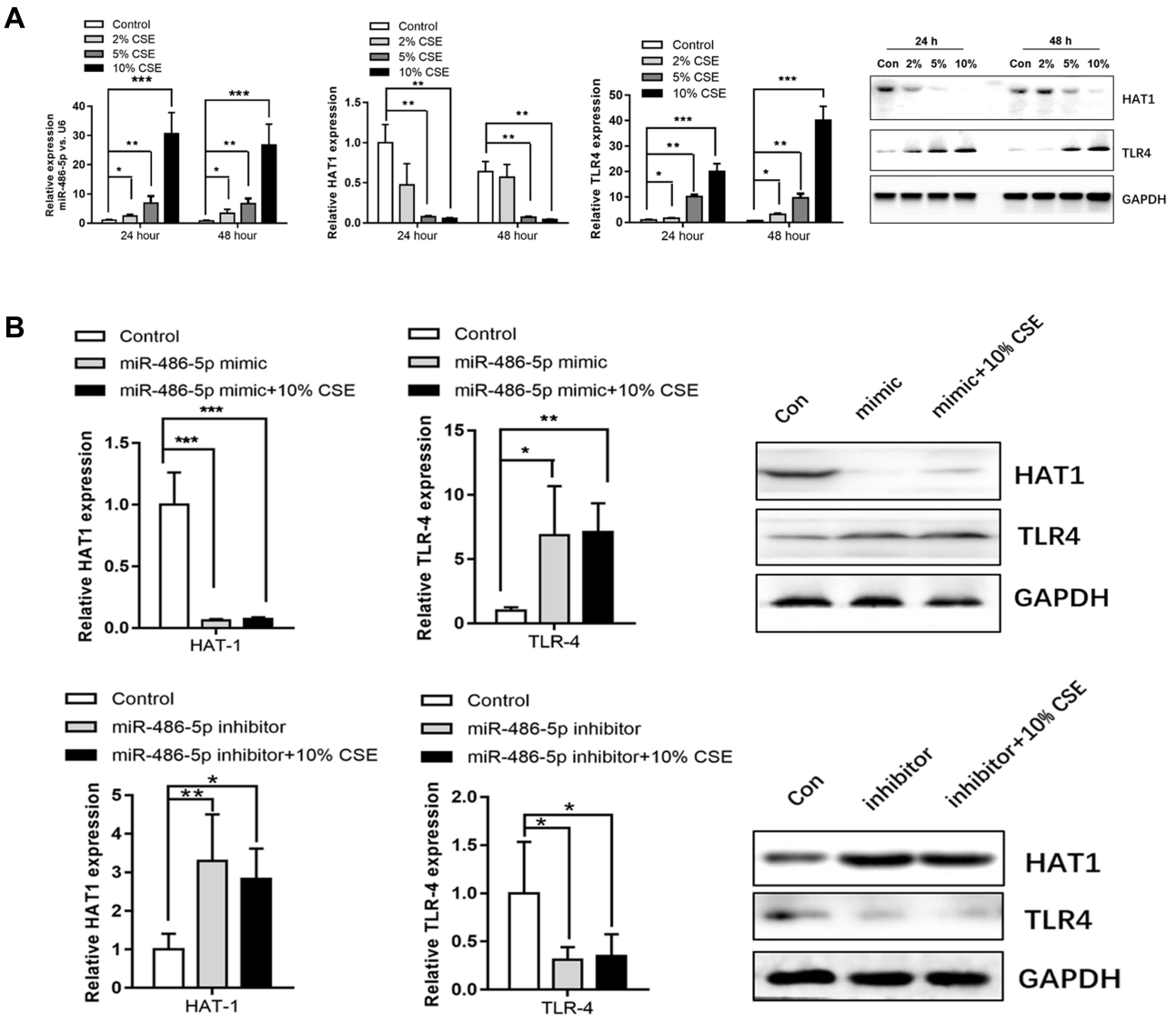

Figure 5 Associations among *miR-486-5p*, *HAT1*, and *TLR4* expression. (**A**) Expression levels of *miR-486-5p*, *HAT1*, and *TLR4* in 2%, 5%, and 10% CSE-treated NR8383 cells, determined using qPCR and Western blotting. (**B**) HAT1 and TLR4 expression after the overexpression or inhibition of *miR-486-5p*. *P < 0.05; **P < 0.01; ***P < 0.001.

the associations between *miR-486-5p* and TLR4 have not been reported. Based on our results, we concluded that *miR-486-5p* regulates *TLR4* expression by targeting *HAT1*.

Here, the results of bioinformatics analysis showed that *HAT1* was a target of *miR-486-5p*. The luciferase reporter assay confirmed the finding that miR-486 could directly bind to the 3′ UTR of *HAT1* to regulate *HAT1* expression. Consistently, Liu et al also found that *miR-486* can directly target *HAT1* to mediate cholesterol efflux in macrophages.[33] Additionally, with si-*HAT1* knockdown and *HAT1* overexpression experiments, we found that *HAT1* negatively regulated the expression of TLR4 and inflammatory cytokines. In recent years, several researchers have focused on the effect of epigenetics in COPD treatment. It has been proposed that epigenetic mechanisms are involved in COPD pathogenesis, and this is a promising therapeutic approach based on epigenetic mark-targeting.[34–36] Histones are highly conserved intranuclear alkaline proteins, and their core modifications can affect transcription, DNA replication, and other cellular processes.[37,38] *HAT1* is a type B histone acetyltransferase associated with the acetylation of newly generated histones.[39] Han et al reported that *HAT1* overexpression promotes lung cancer cell apoptosis by regulating the expression of proteinase-activated receptor 2 and Fas.[40] These data indicate that the regulatory interaction between *HAT1* and *TLR4* may be associated with acetylation.

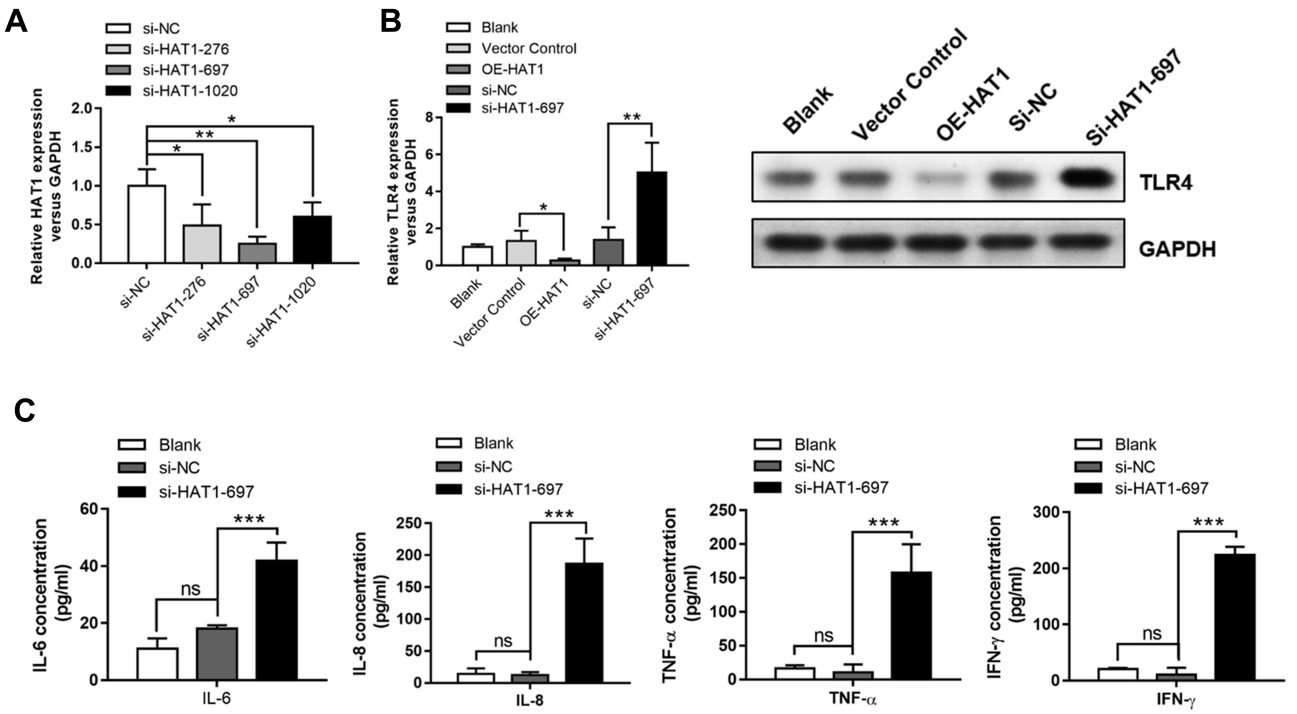

Figure 6 *HAT1* negatively regulated *TLR4* and inflammatory cytokine expression. (**A**) *HAT1* expression in NR8383 cells after transfection with different siRNAs; the most significant inhibition of *HAT1* expression was observed with si-*HAT1* 697. (**B**) The expression of *HAT1* in NR8383 cells after transfection with si-*HAT1* 697 or overexpression of HAT1 using a vector. (**C**) IL-6, IL-8, TNF-α, and IFN-γ levels in NR8383 cells after transfection with si-*HAT1* 697. *P < 0.05; **P < 0.01; ***P < 0.001.

Conclusion

Our results suggest that miRNAs, such as *miR-486-5p*, are differentially expressed in the alveolar macrophages of patients with COPD. Overexpression of *miR-486-5p* may increase the *TLR4*-triggered inflammatory response in COPD patients by targeting *HAT1*. Our findings provide novel targets and a theoretical basis to further investigate COPD pathogenesis, which is crucial for COPD clinical treatment.

Data Sharing Statement

The data used to support the findings of this study are available within the article and the Supplementary Materials.

Ethics Approval and Consent to Participate

This study was conducted under the approval of the Ethics Committee of the Affiliated Huai'an Hospital of Xuzhou Medical University. All enrolled patients signed the informed consent form.

Acknowledgments

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; 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 they have no competing interests.

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