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

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Research

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Abstract

Background: The aim of this study is to investigate the key regulatory miRNA-486-5p and underlying molecular mechanisms in chronic obstructive pulmonary disease (COPD) progression.

Methods: Aberrant miRNA expression in smokers compared to non-smokers and COPD compared to normal was analyzed using microarray datasets and reverse-transcriptase quantitative polymerase chain reaction (qPCR). ELISA assay was used to determine the secretion of inflammatory cytokines in cell supernatants. Inflammatory cytokine expression, including HAT1, TLR4, and miR-486-5p, was determined using qPCR or western blotting. Luciferase reporter assays and fluorescence in situ hybridization were used to confirm the target regulation between miR-486-6p and HAT1.

Results: Our results showed that miR-486-5p was significantly up-regulated in the COPD and smoker groups compared to the control group based on bioinformatics analysis and qPCR validation of alveolar macrophages and peripheral monocytes. miR-218-5p expression significantly correlated with IL-6, IL-8, TNF-α, and IFN-γ expression. Luciferase reporter assays confirmed that miR-486-5p directly targets HAT1, and cellular localization showed that miR-486-5p and HAT1 were highly expressed in the cytoplasm. miR-486-5p overexpression led to significant TLR4 up-regulation and significant HAT1 down-regulation. Inversely, miR-486-5p inhibition led to significant TLR4 down-regulation and significant HAT1 up-regulation. HAT1 knockdown using siRNA significantly increased TLR4, IL-6, IL-8, TNF-α, and IFN-γ expression.

Conclusions: miR-486-5p was differentially expressed in alveolar macrophages of COPD patients. miR-486-5p overexpression might increase the TLR4-triggered inflammatory response in COPD patients by targeting HAT1.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disorder related to ageing and smoking, and it has been a major public health problem due to its high prevalence, morbidity, and mortality [1]. Environment (especially smoking) and genetics are the two major factors that are responsible for COPD etiology. In Western countries, more than 90% of COPD cases are caused by chronic smoking [2, 3]. The immunopathology of COPD is associated with innate and adaptive inflammatory immune responses to chronic smoking [4]. The central feature of COPD is inflammation affecting lung parenchyma and peripheral airways which activates inflammatory cell infiltration, including alveolar macrophages and neutrophils, and other cells. Moreover, the major inflammatory cell types vary with COPD severity [4, 5]. Therefore, a better knowledge of inflammatory responses and corresponding intracellular signaling pathways is essential for COPD drug development and clinical treatment.

Toll-like receptors (TLRs) are a class of evolutionarily conserved innate receptors of the innate immune system that were found to be crucial for host defense by triggering innate and adaptive immunity [6, 7]. TLRs are involved in the pathogenesis of inflammation because they induce inflammatory cytokines and

other endogenous molecules to defend against 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 could promote airway neutrophilia in COPD [10], and tagging single nucleotide polymorphisms in TLR4 was related to sputum inflammatory cell induction and lung function decline [11]. A previous study revealed that TLR4 was highly expressed in bronchial mucosa of severe COPD patients compared to health individuals, and its overexpression was positively correlated with CD4+/CD8+ cell infiltration and airflow obstruction [12]. These results suggested TLR4's 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. Research advances have demonstrated the crucial regulatory roles of miRNAs in many diseases, including COPD [13, 14]. miR-34a was found to be up-regulated in small airway fibroblasts in COPD patients compared to non-smokers, and miR-34a could promote cellular senescence in small airway fibroblasts in COPD patients [15]. miRNA-125a/b overexpression in COPD resulted in heightened airway inflammation by inducing A20 which mediated NF-κB activation, while miRNA-125a/b inhibition decreased inflammatory cytokine induction [16]. Here, we provide evidence that miR-486-5p mediates the TLR4-triggered inflammatory response of alveolar macrophages in COPD. miR-486-5p expression was up-regulated in alveolar macrophages and peripheral monocytes of COPD patients and smokers compared to control, and its expression was positively correlated with IL-6, IL-8, TNF-α, and IFN-γ expression. In addition, miR-486-5p regulated the TLR4-triggered inflammatory response by targeting histone acetyltransferase 1 (HAT1). Our findings provided novel targets and a theoretical basis to further investigate COPD pathogenesis. These are significant steps toward clinical COPD treatment.

Results

Expression level of inflammatory cytokines was elevated with CSE treatment

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

miR-486-5p was up-regulated in COPD

From GSE38974, 17 differentially expressed miRNAs were screened in normal and COPD groups, and miR-486-5p was highly expressed in COPD samples compared to normal samples (Figure 2A). In addition, 45 differentially expressed miRNAs were screened between smoker and non-smoker groups in GSE53519. Notably, miR-486-5p was significantly up-regulated in the small airway epithelium of smokers compared to controls (Figure 2B). miR-486-5p expression was evaluated using qPCR in PAM and peripheral monocytes to confirm bioinformatic data. Consistently, the miR-486-5p level was increased in PAM and peripheral monocytes of COPD patients. Moreover, miR-486-5p was highly expressed in peripheral monocytes of smokers (Figure 2C-D).

CSE resulted in elevated inflammatory cytokines via miR-486-5p

To determine the associations between miR-486-5p expression and inflammatory cytokine levels in cells with CSE treatment, miR-486-5p mimic/inhibitor was used to over-express or inhibit the expression, respectively. Notably, high-expression of miR-486-5p in NR8383 cells led to significant upregulation of IL-6/-8, IFN-γ, and TNF-α, and the upregulation was enhanced in NR8383 cells with CSE treatment (Figure 3A). Inhibition of miR-486-5p in NR8383 cells resulted in significant downregulation of IL-6/-8, IFN-γ, and TNF-α (Figure 3B). We concluded that miR-486-5p plays a role in the regulation of inflammatory responses in COPD.

HAT1 was a target of miR-486-5p

The targeted mRNAs of miR-486-5p were predicted using miRTarBase, TargetScan, miRWalk, and miRPathDB. A total of 11 mRNAs were predicted, including HAT1 (Figure 4A). Luciferase reporter assays and FISH assay were further conducted to confirm the target regulation of miR-486-5p to HAT1. As shown in Figure 4B, luciferase activity of HAT1-WT was remarkably reduced ($P < 0.05$) after co-transfection with miR-486-5p mimic, while the luciferase activity of control and HAT-MUT showed no significant difference (Figure 4B). This indicated that miR-486-5p could bind to the 3'-UTR region of the 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 was decreased in the miR-486-5p mimic and mimic+CSE groups compared to the control group. miR-486-5p inhibition resulted in significant up-regulation of HAT1 in the miR-486-5p inhibitor and inhibitor+CSE groups compared to the control group (Figure 4C).

miR-486-5p regulates TLR4 expression by targeting HAT1

Studies reported that TLR4 could 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 with CSE treatment, while HAT1 expression decreased (Figure 5A). In addition, miR-486-5p overexpression in NR8383 cells led to an obvious increase in TLR4 expression and significantly down-regulated HAT1. Inversely, miR-486-5p inhibition in NR8383 cells resulted in obviously decreased TLR4 expression and significantly up-regulated 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 regulation between HAT1 and TLR4, and si-HAT1-697 showed a stronger knockdown effect (Figure 6A). Notably, HAT1 inhibition by si-HAT1-697 resulted in significant TLR4 up-regulation compared to si-NC and control. Inversely, TLR4 expression was significantly decreased by HAT1 over-expression (Figure 6B). Moreover, IL-6/-8, IFN-γ, and TNF-α expression were significantly increased by inhibiting HAT1 using si-HAT1-697 (Figure 6C). Based on these

results, we suggested that miR-486-5p promoted 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 reported that some miRNAs could serve as important regulators in the molecular mechanisms of COPD [22, 23]. Identification of aberrant miRNA expression in COPD could help to better understand the underlying mechanisms. Studies reported that miRNAs were abnormally expressed in COPD patients and smokers compared to normal patients. For instance, Paschalaki et al. suggested that decreased miR-126 expression contributed to DNA damage in smokers and COPD patients [24]. Conickx et al. suggested that miR-218-5p expression decreased in COPD patients and smokers without airflow limitation, and its expression showed a significant relationship with airway obstruction [23]. Here, miR-486-5p was aberrantly expressed in COPD and smokers compared to non-smokers without COPD based on bioinformatics analysis on a microarray dataset. This was consistent with miR-486-5p expression in PAM and peripheral monocytes of COPD patients and smokers. This suggested that miR-486-5p was involved in COPD progression.

miR-486 is an intragenic miRNA located within Ankyrin 1 [25]. Studies have reported that aberrant miR-486-5p expression could affect the occurrence and progression of many diseases [26, 27]. Notably, the effect of miR-486-5p is controversial because it was found to serve as a suppressor or oncogene in studies [28, 29]. In addition, we saw that miR-486-5p's function had not been explored in COPD. Here, the results revealed that miR-486-5p expression was significantly correlated with IL-6/-8, IFN-γ, and TNF-α expression. Therefore, we suggested that miR-486-5p might play roles in mediating inflammatory response. Chai et al. had reported that increased miR-486-5p levels repressed the lipopolysaccharide-induced expression of inflammatory cytokines like IL-1β, IL-6, and TNF-α [30]. TLRs were demonstrated to function in inflammatory pathogenesis because they could induce inflammatory cytokines and other endogenous molecules to defend against pathogenic microbial infection [8, 9]. Recently, miRNAs were found to serve crucial regulatory roles in TLR signaling. For example, Shen et al. revealed that miR-149-3p could affect the inflammatory response, especially IL-1β and TNF-α expression, by regulating TLR-4/NF-κB signaling pathways in COPD patients [31]. Lai et al. suggested that miR-92a could regulate the TLR4-triggered inflammatory response in macrophages via JNK/c-Jun signaling activated by mitogen-activated protein kinase 4 [32]. However, the associations between miR-486-5p and TLR4 have not been reported. Based our results, we concluded that miR-486-5p regulates TLR4 expression by targeting HAT1.

Here, the results of bioinformatic 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 could directly target HAT1 to mediate

cholesterol efflux in macrophages [33]. Additionally, we found that HAT1 negatively regulated the expression of TLR4 and inflammatory cytokines based on si-HAT1 knockdown or HAT1 overexpression. In recent years, a growing group of researchers has focused on the effect of epigenetics in COPD treatment. It was proposed that epigenetic mechanisms are involved in COPD pathogenesis, and this was a promising therapeutic approach based on epigenetic mark targeting [34-36]. Histones are highly conserved intra-nuclear 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. had indicated that HAT1 overexpression could promote lung cancer cell apoptosis by regulating the expression of proteinase-activated receptor 2 and Fas [40]. These data indicated that the regulation between HAT1 and TLR4 expression might be associated with acetylation.

Conclusion

Our results suggested that miRNAs are differentially expressed in alveolar macrophages of COPD patients, like miR-486-5p overexpression. Overexpression of miR-486-5p might increase the TLR4-triggered inflammatory response in COPD patients by targeting HAT1. Our findings provided novel targets and a theoretical basis to further investigate COPD pathogenesis, and this is significant for COPD clinical treatment.

Abbreviations

cDNA: complementary DNA; COPD: chronic obstructive pulmonary disease; CSE: Cigarette smoke extract; HAT1: Histone acetyltransferase 1; IFN-γ: Interferon-γ; IL-6: Interleukin-6; IL-8: Interleukin-8; qPCR: reverse-transcriptase quantitative polymerase chain reaction; TLR4: Toll-like receptor 4; TNF-α: Tumor necrosis factor-α; PAM: Alveolar macrophages

Declarations

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. And All enrolled patients signed the informed consent.

Consent for publication

Not applicable.

Availability of data and material

The data used to support the findings of this study are included within the article and supplementary tables.

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Competing interests

We declare that they have no competing interests.

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Author's contributions

YLZ and YFW conceived and designed the experiments. JZ, ZNX and LHK performed most of the experiments. HG and YMZ helped to analyzed the data. JZ and ZNX drafted the manuscript. All authors read and approved the final manuscript.

Materials And Methods

Cigarette smoke extract (CSE)

CSE preparation was done based on the method below. Briefly, three cigarettes without filters (0.9mg flue gas nicotine, 11mg coke, 14mg flue gas carbon monoxide) were collected using a negative pressure suction device and poured into a flask with 3 mL PBS. After shaking well, the suspension was filtered using 0.22 m filter membrane to filter bacteria. To ensure the same CSE concentration, the absorbance value was determined at the optimal absorption wavelength (270-280nm), and the absorbance value of the prepared CSE was the same in each preparation. The CSE concentrate was diluted using 10% FBS containing PRMI-1640 culture medium to obtain 2%, 5%, and 10% CSE. Notably, the CSE were prepared 30 min before use to ensure the effectiveness of 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 CO_2 (5%). Cells were placed in 6-well plates with cell density of $1*10^6$ cells per well and cultured in serum-free F12K medium overnight. Cells were treated with CSE for 24 h and 48 h.

Quantitative real-time PCR

Total RNA extraction was performed utilizing Trizol Reagent in light of the instructions of supplier. After determining the RNA concentration and quality, complementary DNA (cDNA) was synthesized using a

First Strand cDNA Synthesis Kit (Sangon biotech, China). Real-time qPCR was performed to validate gene expression using 2× SYBR Green PCR Master Mix (Sangon biotech, China) on a 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 were shown in supplemental table 1.

Western blotting

Total protein extraction was conducted according to the M-PER Mammalian Protein Extraction Reagent instructions before separating proteins using a 10% SDS-polyacrylamide gel. The proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore) using Semi-Dry Cell at 30 mA for 60 min. 1× Ponseau S solution was used to dye and mark the protein marker spots. Blocking Buffer (1×TBS, 0.1% Tween-20, and 5% w/v nonfat 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 before incubating with HRP-secondary antibodies (1:2000, Jackson) for 2 h at 37°C. After washing five times with 1× TBST, the membrane was exposed with SuperSignal West Pico Chemiluminent Substrates, and gray scanning was preformed using a Gel-Pro Analyzer.

Luciferase reporter assays

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

ELISA assay

After treating with CSE, the cell supernatants were collected and analyzed with ELISA kits (Sangon biotech, China) based on the supplier’s instructions.

MiRNA mimics/inhibitors and small interfering RNA

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

Fluorescence in situ hybridization

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 dehydrating.

The fixed cells were prehybridized in hybridization buffer (formamide, 50 mM Tris-HCl, 5 mol NaCl, and 0.05% SDS) based on the method previously described. 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 using 4°C PBS buffer. Cells were observed under a fluorescence microscope with a 360 nm excitation wavelength and a 460nm emission wavelength filter.

Clinical sample collection

The bronchoalveolar lavage fluid was collected from 14 normal individuals and 36 COPD patients. Next, alveolar macrophages (PAM) were isolated. In brief, 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 RPMI1640 at 37°C in an incubator with 5% CO_2 and 100% humidity for 2-3h. Non-adherent cells were removed, and the cells adhering to the culture dish wall were PAM. Peripheral blood was collected from 128 participants (No-smoker: n=33; smoker: 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 in January 2019, 1 solstice, June 30, 2019 in the respiratory department and physical examination center at the Affiliated Huai'an Hospital of Xuzhou Medical University. Written informed consent was obtained from participants before collecting samples. 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 miRNAs expression data of small airway epithelium from nine non-smokers 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 SPSS19.0 and GraphPad Prism 7. Mean ± standard deviation (SD) was selected to present all the results. Student's t-test was used to compare the data between the two groups. Variance analysis was performed to compare multiple groups. One-way ANOVA 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 pair-wise comparison among multiple groups. Differences were statistically significant if $P < 0.05$.

References

1. López-Campos JL, Tan W, Soriano JB: **Global burden of COPD.***Respirology* 2016, **21:**14-23.
2. Tan WC, Bourbeau J, Aaron SD, Zhou G, Maltais F, Hernandez P, Fitzgerald JM, Marciniuk DD, Walker BL, Sin DD: **Global initiative for chronic obstructive lung disease 2017 classification and lung function decline in chronic obstructive pulmonary disease.***American journal of respiratory and critical care medicine* 2018, **197:**670-673.
3. Jindal SK: **Chronic obstructive pulmonary disease in non-smokers-Is it a different phenotype?***The Indian journal of medical research* 2018, **147:**337.
4. Caramori G, Casolari P, Barczyk A, Durham AL, Di Stefano A, Adcock I: **COPD immunopathology.***Seminars in Immunopathology* 2016, **38:**497-515.
5. Barnes PJ: **Inflammatory mechanisms in patients with chronic obstructive pulmonary disease.***Journal of Allergy and Clinical Immunology* 2016, **138:**16-27.
6. Kawai T, Akira S: **The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors.***Nature immunology* 2010, **11:**373.
7. Kumar H, Kawai T, Akira S: **Toll-like receptors and innate immunity.***Biochemical and biophysical research communications* 2009, **388:**621-625.
8. Drexler SK, Foxwell BM: **The role of toll-like receptors in chronic inflammation.***The international journal of biochemistry & cell biology* 2010, **42:**506-518.
9. Achek A, Yesudhas D, Choi S: **Toll-like receptors: promising therapeutic targets for inflammatory diseases.***Archives of pharmacal research* 2016, **39:**1032-1049.
10. Pace E, Giarratano A, Ferraro M, Bruno A, Siena L, Mangione S, Johnson M, Gjomarkaj M: **TLR4 upregulation underpins airway neutrophilia in smokers with chronic obstructive pulmonary disease and acute respiratory failure.***Human immunology* 2011, **72:**54-62.
11. Budulac SE, Boezen HM, Hiemstra PS, Lapperre TS, Vonk JM, Timens W, Postma DS, group Gs: **Toll-like receptor (TLR2 and TLR4) polymorphisms and chronic obstructive pulmonary disease.***PloS one* 2012, **7:**e43124-e43124.
12. Di Stefano A, Ricciardolo FL, Caramori G, Adcock IM, Chung KF, Barnes PJ, Brun P, Leonardi A, Andò F, Vallese D: **Bronchial inflammation and bacterial load in stable COPD is associated with TLR4 overexpression.***European Respiratory Journal* 2017, **49**.
13. Sauler M, Nouws J, Feng W, Lee P: **MicroRNA Regulation of Cell Fate in the Pathogenesis of COPD.** In *C63 MECHANISTIC STUDIES IN COPD.* American Thoracic Society; 2019: A5375-A5375
14. Tasena H, Faiz A, Timens W, Noordhoek J, Hylkema MN, Gosens R, Hiemstra PS, Spira A, Postma DS, Tew GW: **microRNA–mRNA regulatory networks underlying chronic mucus hypersecretion in COPD.***European Respiratory Journal* 2018, **52:**1701556.

15. Wrench C, Baker J, Fenwick P, Donnelly L, Barnes P: **MicroRNA-34a drives small airway fibroblast cellular senescence in COPD.***European Respiratory Journal* 2017, **50:**OA289.
16. Hsu AC, Dua K, Starkey MR, Haw T-J, Nair PM, Nichol K, Zammit N, Grey ST, Baines KJ, Foster PS: **MicroRNA-125a and-b inhibit A20 and MAVS to promote inflammation and impair antiviral response in COPD.***JCI insight* 2017, **2**.
17. He W, Rebello O, Savino R, Terracciano R, Schuster-Klein C, Guardiola B, Maedler K: **TLR4 triggered complex inflammation in human pancreatic islets.***Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 2019, **1865:**86-97.
18. Liu C, Tang X, Zhang W, Li G, Chen Y, Guo A, Hu C: **6-Bromoindirubin-3'-Oxime Suppresses LPS-Induced Inflammation via Inhibition of the TLR4/NF-κB and TLR4/MAPK Signaling Pathways.***Inflammation* 2019, **42:**2192-2204.
19. Hogg JC, Paré PD, Hackett T-L: **The contribution of small airway obstruction to the pathogenesis of chronic obstructive pulmonary disease.***Physiological reviews* 2017, **97:**529-552.
20. Tuder RM: **Bringing light to chronic obstructive pulmonary disease pathogenesis and resilience.***Annals of the American Thoracic Society* 2018, **15:**S227-S233.
21. Boucherat O, Morissette MC, Provencher S, Bonnet S, Maltais F: **Bridging lung development with chronic obstructive pulmonary disease. Relevance of developmental pathways in chronic obstructive pulmonary disease pathogenesis.***American journal of respiratory and critical care medicine* 2016, **193:**362-375.
22. Huang X, Zhu Z, Guo X, Kong X: **The roles of microRNAs in the pathogenesis of chronic obstructive pulmonary disease.***International immunopharmacology* 2019, **67:**335-347.
23. Conickx G, Mestdagh P, Avila Cobos F, Verhamme FM, Maes T, Vanaudenaerde BM, Seys LJ, Lahousse L, Kim RY, Hsu AC: **MicroRNA profiling reveals a role for microRNA-218-5p in the pathogenesis of chronic obstructive pulmonary disease.***American journal of respiratory and critical care medicine* 2017, **195:**43-56.
24. Paschalaki KE, Zampetaki A, Baker JR, Birrell MA, Starke RD, Belvisi MG, Donnelly LE, Mayr M, Randi AM, Barnes PJ: **Downregulation of microRNA-126 augments DNA damage response in cigarette smokers and patients with chronic obstructive pulmonary disease.***American journal of respiratory and critical care medicine* 2018, **197:**665-668.
25. Oh HK, Tan AL-K, Das K, Ooi C-H, Deng N-T, Tan IB, Beillard E, Lee J, Ramnarayanan K, Rha S-Y: **Genomic Loss of miR-486 Regulates Tumor Progression and the OLFM4 Antiapoptotic Factor in Gastric Cancer.***Clinical Cancer Research An Official Journal of the American Association for Cancer Research*, **17:**2657-2667.
26. Fu SJ, Chen J, Ji F, Ju WQ, Zhao Q, Chen MG, Guo ZY, Wu LW, Ma Y, Wang DP: **MiR-486-5p negatively regulates oncogenic NEK2 in hepatocellular carcinoma.** 2017, **8:**52948-52959.
27. Shi L, Liu S, Zhao W, Shi J: **miR-483-5p and miR-486-5p are down-regulated in cumulus cells of metaphase II oocytes from women with polycystic ovary syndrome.***Reproductive Biomedicine Online* 2015, **31:**565-572.

28. Shao Y, Shen YQ, Li YL, Liang C, Ma Z-L: **Direct repression of the oncogene CDK4 by the tumor suppressor miR-486-5p in non-small cell lung cancer.***Oncotarget* 2016, **7:**34011-34021.
29. Gao ZJ, Yuan W-d, Yuan J-q, Yuan K, Wang Y: **miR-486-5p functions as an oncogene by targeting PTEN in non-small cell lung cancer.***Pathology Research & Practice***:**S0344033818301109.
30. Xingyu, Chai, Haipeng, Si, Jiang, Song, Yanxue, Chong, Jing, Wang: **miR-486-5p Inhibits Inflammatory Response, Matrix Degradation and Apoptosis of Nucleus Pulposus Cells through Directly Targeting FOXO1 in Intervertebral Disc Degeneration.***Cellular Physiology and Biochemistry* 2019, **52:**109-118.
31. Shen W, Liu J, Zhao G, Fan M, Song G, Zhang Y, Weng Z, Zhang Y: **Repression of Toll-like receptor-4 by microRNA-149-3p is associated with smoking-related COPD.***International Journal of Chronic Obstructive Pulmonary Disease*, **Volume 12:**705-715.
32. Lai L, Song Y, Liu Y, Chen Q, Han Q, Chen W, Pan T, Zhang Y, Cao X, Wang Q: **MicroRNA-92a Negatively Regulates Toll-like Receptor (TLR)-triggered Inflammatory Response in Macrophages by Targeting MKK4 Kinase.***Journal of Biological Chemistry*, **288:**7956-7967.
33. Liu D, Zhang M, Xie W, Lan G, Cheng HP, Gong D, Huang C, Lv YC, Yao F, Tan YL: **MiR-486 regulates cholesterol efflux by targeting HAT1.** 2016, **472:**418-424.
34. Schamberger AC, Mise N, Meiners S, Eickelberg O: **Epigenetic mechanisms in COPD: Implications for pathogenesis and drug discovery.***Expert Opinion on Drug Discovery* 2014, **9:**609-628.
35. Wu D-D, Song J, Bartel S, Krauss-Etschmann S, Rots MG, Hylkema MN: **The potential for targeted rewriting of epigenetic marks in COPD as a new therapeutic approach.***Pharmacology & therapeutics* 2018, **182:**1-14.
36. Adcock IM: **Role of epigenetic modifications in pathology of COPD.***Tanaffos* 2017, **16:**S2.
37. Lawrence M, Daujat S, Schneider R: **Lateral thinking: how histone modifications regulate gene expression.***Trends in Genetics* 2016, **32:**42-56.
38. Stillman B: **Histone modifications: insights into their influence on gene expression.***Cell* 2018, **175:**6-9.
39. Parthun M: **Hat1: the emerging cellular roles of a type B histone acetyltransferase.***Oncogene* 2007, **26:**5319-5328.
40. Han N, Shi L, Guo Q, Sun W, Yu Y, Yang L, Zhang X, Zhang M: **HAT1 induces lung cancer cell apoptosis via up regulating Fas.***Oncotarget* 2017, **8:**89970.
41. Chou C-H, Shrestha S, Yang C-D, Chang N-W, Lin Y-L, Liao K-W, Huang W-C, Sun T-H, Tu S-J, Lee W-H: **miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions.***Nucleic acids research* 2018, **46:**D296-D302.
42. Agarwal V, Bell GW, Nam J-W, Bartel DP: **Predicting effective microRNA target sites in mammalian mRNAs.***elife* 2015, **4:**e05005.
43. Dweep H, Gretz N: **miRWalk2. 0: a comprehensive atlas of microRNA-target interactions.***Nature methods* 2015, **12:**697-697.\
44. Backes C, Kehl T, Stöckel D, Fehlmann T, Schneider L, Meese E, Lenhof H-P, Keller A: **miRPathDB: a new dictionary on microRNAs and target pathways.***Nucleic acids research* 2016**:**gkw926.

Figures

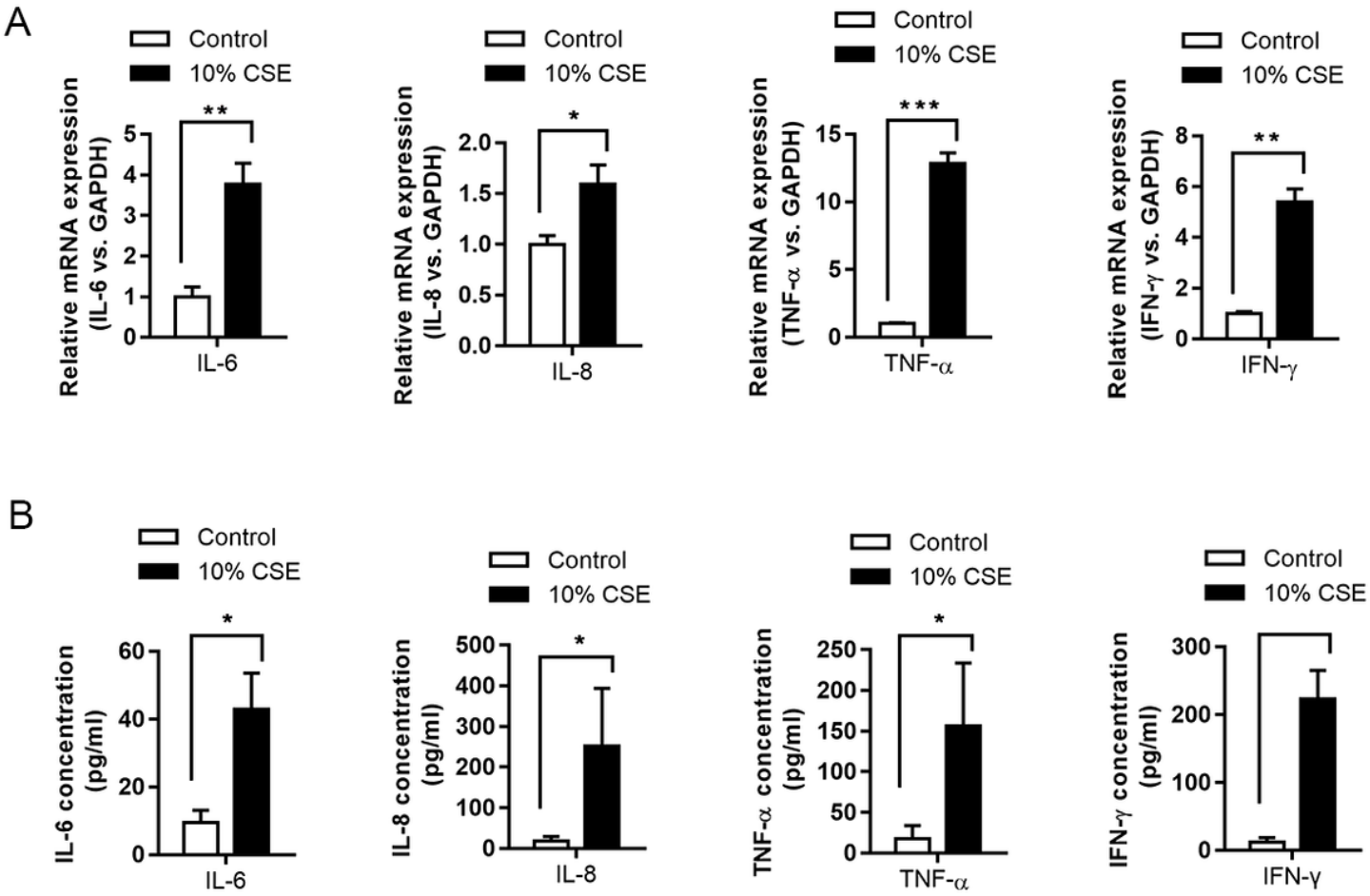


Figure 1

Level of inflammatory cytokines in NR8383 cells with CSE treatment. The mRNA expression of IL-6, IL-8, TNF-α, and IFN-γ in 10% CSE-treated NR8383 cells determined by qPCR (A). The level of IL-6, IL-8, TNF-α, and IFN-γ in cell supernatants determined by ELISA kits (B). *P < 0.05; **P < 0.01; ***P < 0.001.

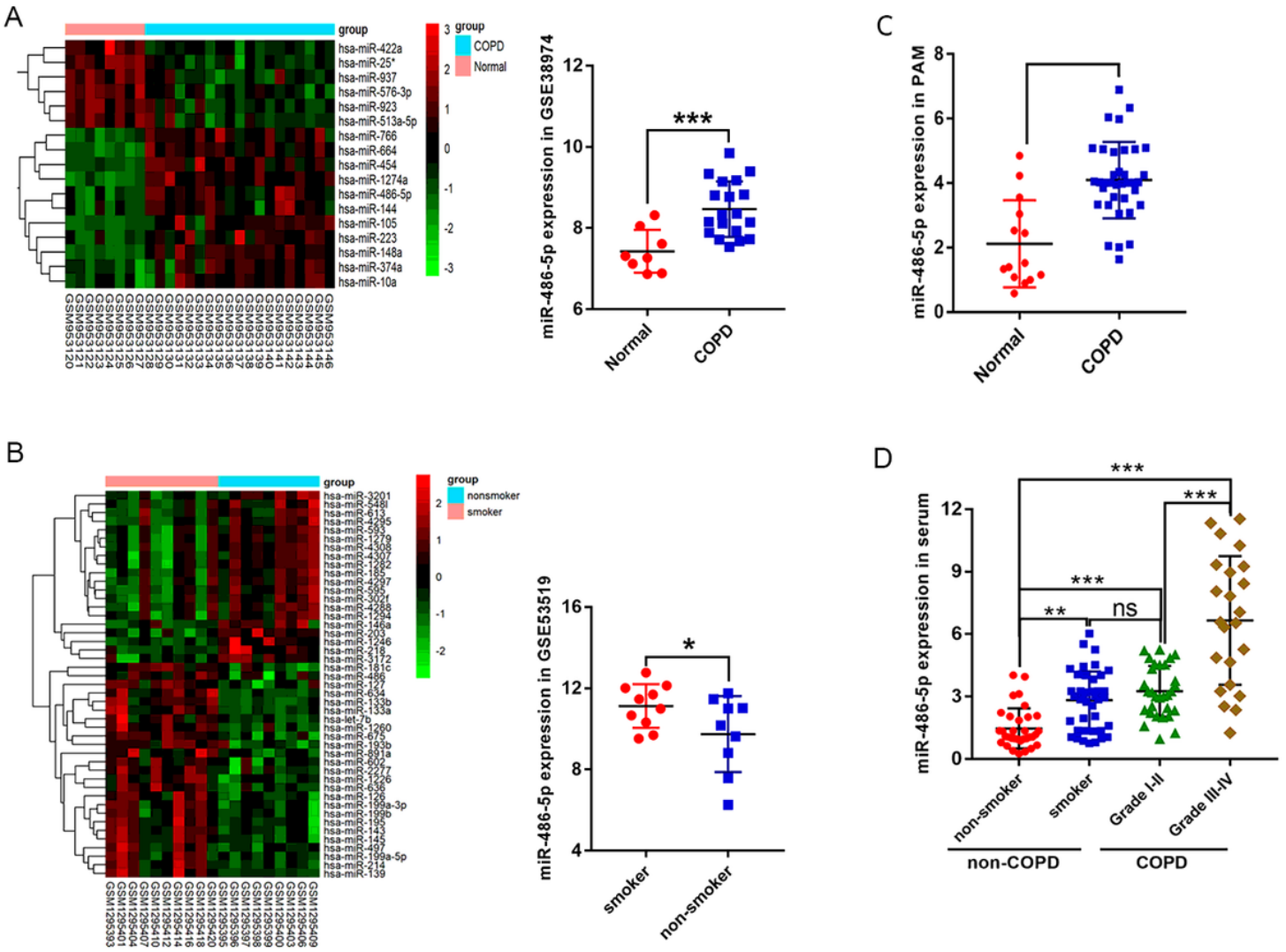


Figure 2

Screening and validation of differentially expressed miRNAs. Heat maps of differentially expressed miRNAs in COPD vs. normal based on the GSE38974 dataset and miR-486-5p expression in the GSE38974 dataset (A). Heat maps of differentially expressed miRNAs in smokers vs. non-smokers 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 by qPCR (C). miR-486-5p expression in peripheral monocytes in smokers (n=42) vs. non-smokers (n=33) and in COPD I-II (n=30) vs. COPD III-IV (n=23) determined by qPCR (D). *P < 0.05; **P < 0.01; ***P < 0.001.

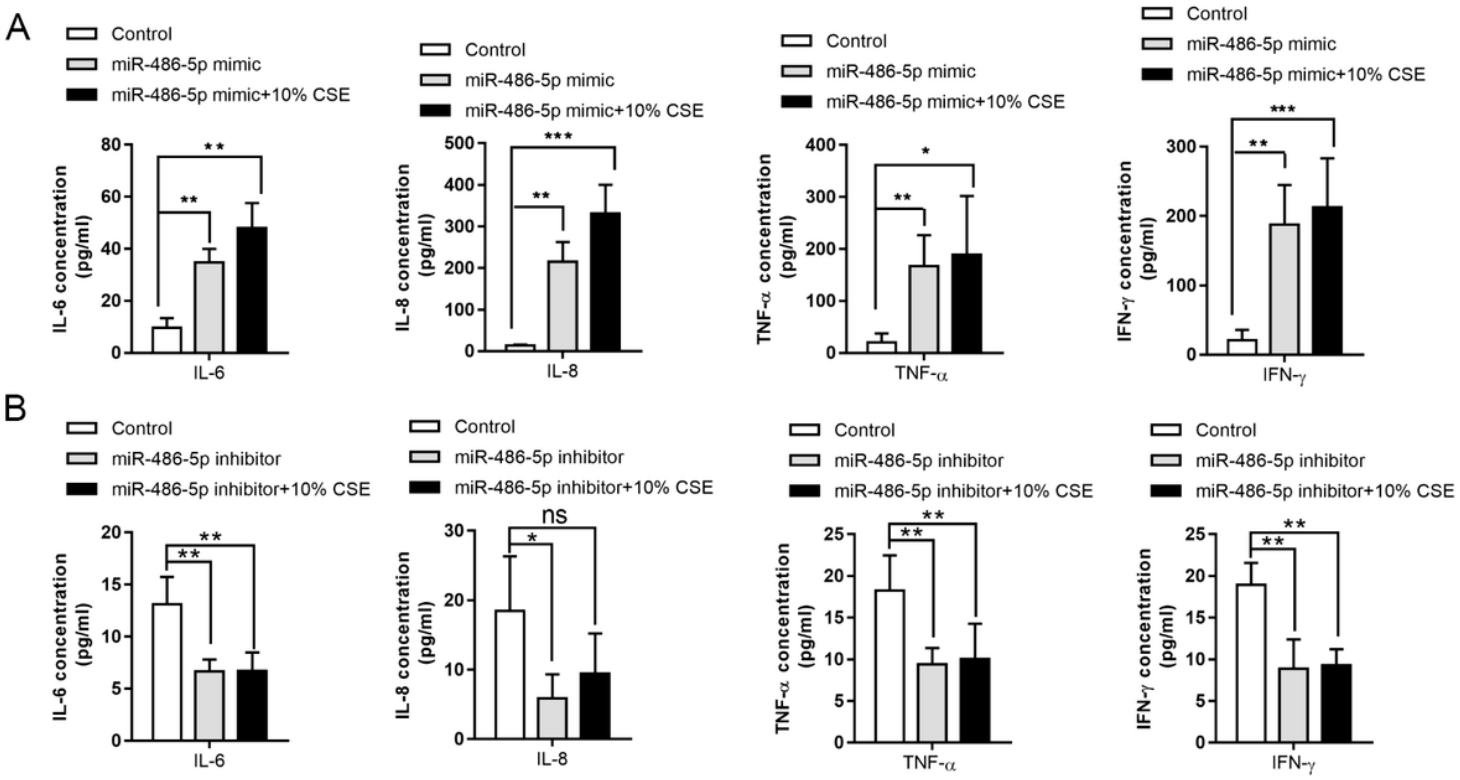


Figure 3

Associations between inflammatory cytokines and miR-486-5p expression. IL-6, IL-8, TNF-α, and IFN-γ levels in cell supernatants determined by ELISA kits. (A) IL-6, IL-8, TNF-α, and IFN-γ levels in cells after transferring miR-486-5p mimic. (B) IL-6, IL-8, TNF-α, and IFN-γ levels in cells after transferring miR-486-5p inhibitor. *P < 0.05; **P < 0.01; ***P < 0.001.

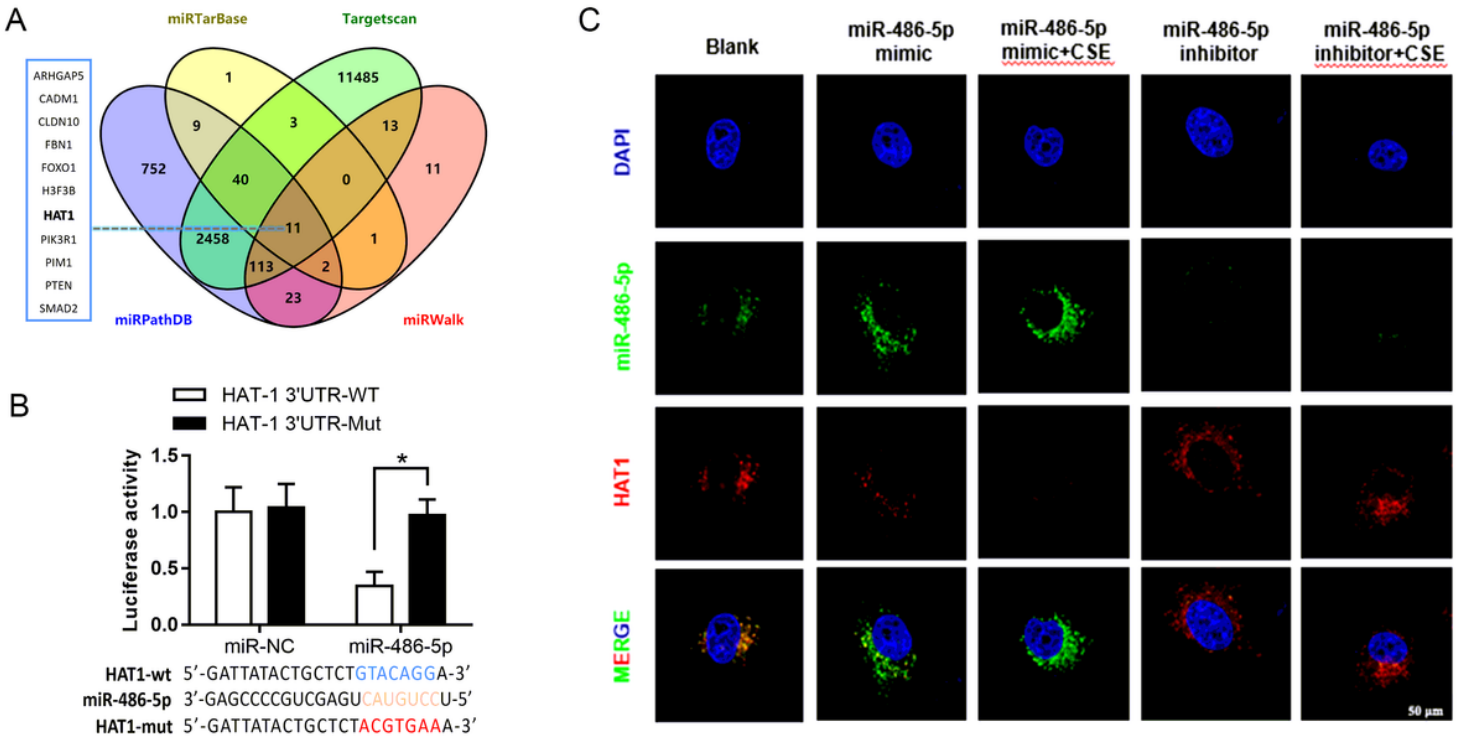
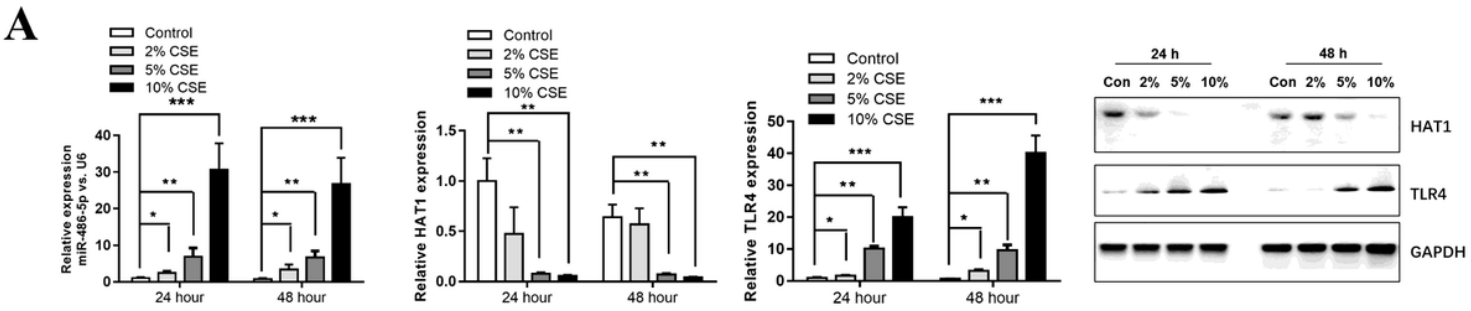


Figure 4

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



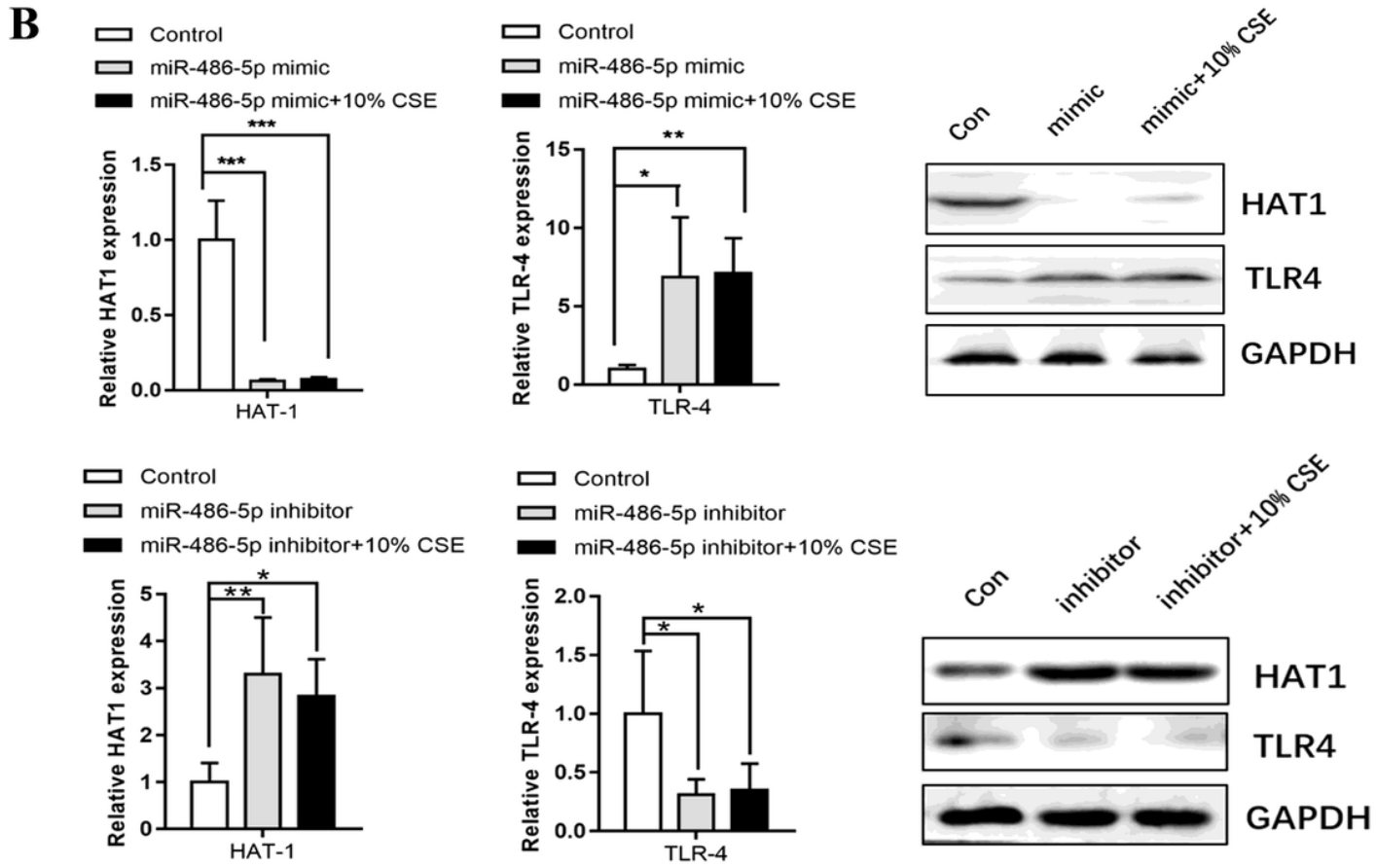


Figure 5

Associations among miR-486-5p, HAT1, and TLR4 expression. (A) Expression of miR-486-5p, HAT1, and TLR4 in 2%, 5%, and 10% CSE-treated NR8383 cells determined by qPCR and western blotting, respectively. (B) HAT1 and TLR4 expression after overexpressing or inhibiting miR-486-5p. *P < 0.05; **P < 0.01; ***P < 0.001.

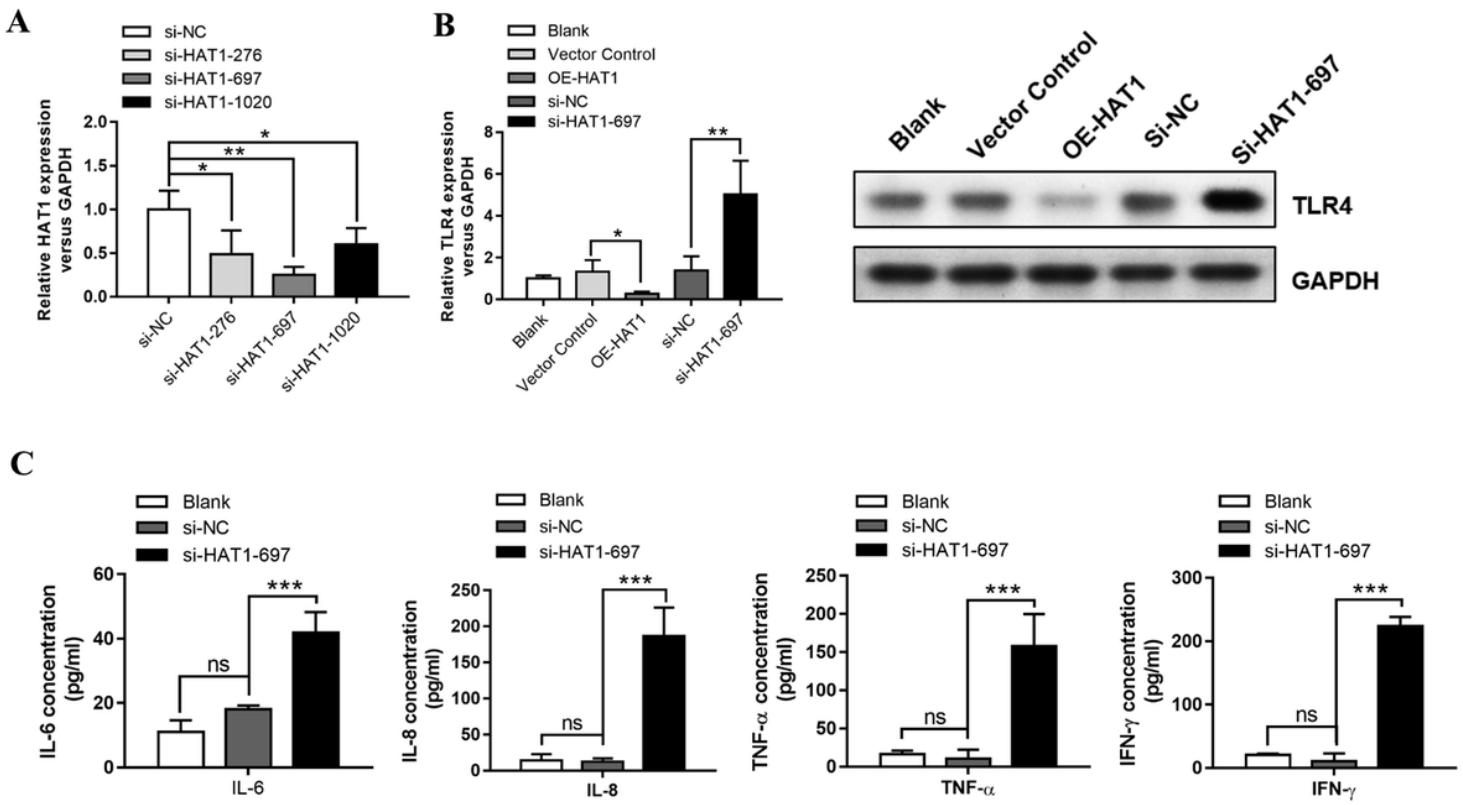


Figure 6

HAT1 negatively regulated TLR4 and inflammatory cytokine expression. (A) HAT1 expression in NR8383 cells after transferring different siRNAs, with the more significant HAT1 expression inhibition resulting from si-HAT1 697. (B) The expression of HAT1 in NR8383 cells after transferring si-HAT1 697 or overexpressing HAT1 by vector. (C) IL-6, IL-8, TNF-α, and IFN-γ levels in NR8383 cells after transferring si-HAT1 697. *P < 0.05; **P < 0.01; ***P < 0.001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS2.docx
- TableS1.doc