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ORIGINAL RESEARCH

miR-335-5p Predicts Elevated Chronic Obstructive Pulmonary Disease (COPD) Susceptibility and Its Role in Human Bronchial Epithelial Cells Injury

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Purpose: Chronic obstructive pulmonary disease (COPD) suffers from high prevalence, disability and mortality rates and a heavy economic burden. miR-335-5p takes part in multiple respiratory diseases such as pulmonary fibrosis, whereas its study in COPD has not been reported. The aim of our research was to explore miR-335-5p in predicting elevated COPD susceptibility and in human bronchial epithelial cells injury.

Patients and Methods: qRT-PCR was performed to examine miR-335-5p levels in serum and cells. ROC curve and logistic regression analyses were utilized to evaluate the predictive capacity of miR-335-5p for COPD susceptibility. Pearson correlation was used to assess the association of miR-335-5p with TNF-α, IL-6, FEV1, and FEV1/FVC. Human bronchial epithelial cells were exposed to cigarette smoke extract (CSE) conditions to simulate cell injury. Cell proliferation, apoptosis, inflammatory response and oxidative stress-related factors were assayed by CCK8, flow cytometry and ELISA, respectively.

Results: miR-335-5p is reduced on COPD patients. ROC curve recommended that miR-335-5p has high sensitivity (88.9%) and specificity (80.0%) to distinguish COPD from healthy individuals. Logistic regression showed that reduced miR-335-5p predicted elevated COPD susceptibility. Moreover, miR-335-5p was significantly negatively related to TNF-α and IL-6 and positively related to FEV1, and FEV1/FVC in COPD patients. Cellular experiments revealed that CSE treatment decreased miR-335-5p expression, repressed cell proliferation, facilitated apoptosis, raised TNF-α, IL-6, ROS, and MDA levels, and reduced SOD levels. miR-335-5p overexpression facilitated cell proliferation, suppressed apoptosis, diminished TNF-α, IL-6, ROS, and MDA levels, and elevated SOD levels, whereas knockdown of miR-335-5p reversed this trend.

Conclusion: Downregulation of miR-335-5p increased COPD susceptibility and negatively correlated with inflammatory factors. Overexpression of miR-335-5p alleviated CSE-induced injury to human bronchial epithelial cells, which suggested that miR-335-5p may be a potential target for COPD treatment.

Keywords: chronic obstructive pulmonary disease, susceptibility, human bronchial epithelial cells, miR-335-5p

Introduction

Chronic obstructive pulmonary disease (COPD) is a widespread and frequent disease, which is a chronic inflammation of the airways caused by repeated exposure to harmful environments.[1] Progressive and irreversible airflow limitation as well as lung damage are features of COPD.[2] The main symptoms include shortness of breath, chronic cough and sputum, which are progressive and without intervention will gradually deteriorate over time, thus seriously affecting the quality of life.[3,4] According to the findings of the 2018 China pulmonary health study, it was pointed out for the first time that the number of COPD patients in China is now nearly 100 million, with a prevalence rate of 8.6% among adults over 20 years old and 13.7% among those over 40 years old.[5] The high morbidity and mortality rates of COPD are gradually attracting

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attention. Therefore, timely diagnosis and therapy are critical to slowing the advancement of COPD and reducing its susceptibility.

miRNAs are prevalent in multiple body fluids and have relatively stable properties.[6] Therefore, miRNAs have great potential as biomarkers. Since their discovery, miRNAs have been broadly researched in several systems, including respiratory, digestive, and urinary.[7–9] In the respiratory system, the involvement of miRNAs has been found in the development of diseases such as asthma, pulmonary fibrosis and lung cancer.[10–12] Evidence suggests that dysregulation of miRNA expression profiles affect biological processes such as oxidative stress and inflammation in COPD, contributing to the occurrence and progression of the disease.[13] For example, miR-21 and miR-186-5p were involved in COPD development by regulating inflammation-related factors.[14,15] miR-335-5p was found to exert a regulatory function in multiple respiratory diseases. For example, in smokers' lung fibroblasts, it was found that miR-335-5p level was markedly lower than quitters.[16] Dysregulation of miR-335-5p discovered in studies of epigenetic regulation of post-infectious bronchiolitis obliterans.[17] Importantly, miR-335-5p was identified as downregulated in studies examining smokers and COPD.[18] However, the miR-335-5p influence on COPD susceptibility and functions it exerts has not been reported.

Generally, human bronchial epithelial cells are used for in vitro studies of COPD. Firstly, bronchial epithelial cells are the primary target of exposure to external stimuli such as cigarette smoke and air pollutants, and they are the pathological starting point for lung diseases such as COPD. Secondly, the abnormal function of bronchial epithelial cells is the core factor for respiratory symptoms such as coughing and excessive phlegm in patients with COPD. In addition, in vitro bronchial epithelial cell models can accurately simulate disease microenvironments such as CSE-induced injury, facilitating quantitative analysis of miRNA-regulated molecular mechanisms by cell transfection.

For our study, we evaluated the impact of miR-335-5p on COPD susceptibility by measuring its expression in COPD patients. The association of miR-335-5p with inflammatory factors in COPD patients was analyzed to explore whether miR-335-5p dysregulation affects COPD progression by influencing the inflammatory process. Furthermore, the function of miR-335-5p on human bronchial epithelial cells was experimentally verified to analyze the role it plays in cell injury. This research may provide direction in searching for new biomarkers and potential therapeutic targets for COPD.

Materials and Methods

Participants

The study retrospectively included COPD patients recruited at the Respiratory Medicine Outpatient Clinic of Zhangzhou Affiliated Hospital of Fujian Medical University from March 2019 to May 2022. The COPD patients were screened strictly based on inclusion and exclusion criteria and 117 patients were set up as COPD group. And in our hospital's physical examination center, 100 cases of healthy individuals were chosen as healthy group.

Inclusion criteria for the COPD group were a forced expiratory volume in first second/ forced vital capacity (FEV_1 /FVC) after inhalation of a bronchodilator < 70% and age ≥ 40 years. Exclusion criteria included having other respiratory diseases such as asthma, pneumonia, and pulmonary fibrosis; co-morbidities such as tumors and autoimmune diseases; and pregnant and breastfeeding women. The healthy group included participants who were matched to COPD group in terms of basic characteristics such as age and gender and without family history of COPD.

This study was performed in line with the principles of the Declaration of Helsinki. The research was approved by the Clinical Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University, and all participants signed an informed consent.

Collection of Blood Samples and Data

The COPD and healthy group collected 5 mL venous blood samples after fasting for 8 h and placed them in serum tubes. Centrifuge at 4000 rpm/min for 5 min at 4°C within 2 h. After centrifugation, the supernatant was gathered into new centrifuge tubes and numbered accordingly, as well as the serum samples were subsequently saved at −80°C for spare use.

Basic information including age, gender, body mass index (BMI), smoking history, and pulmonary function test data (FEV_1 and FEV_1/FVC) were retrieved and gathered from all participants in the study through electronic medical records.

Extraction of Peripheral Blood Mononuclear Cells

Venous blood was collected into anticoagulant tubes containing heparin sodium, and the blood samples were mixed and diluted with an equal volume of PBS. Then, it was slowly added to the Ficoll-Hypaque separation (Cytiva, USA) to avoid interfacial mixing. It was centrifuged at 550 g for 30 min at room temperature and the greyish-white peripheral blood mononuclear cells layer was aspirated using a pasteurized tube. PBS was added and centrifuged at 800 g for 10 min, supernatant was removed, and the precipitate was used for subsequent experiments.

Preparation of Cigarette Smoke Extract (CSE)

A three-way tube was connected to 1 lit cigarette and a syringe, and the aspirated smoke was dissolved in the pre-prepared DMEM medium (Gibco, USA). The medium is rapidly filtered through a filter with a 0.22 μm membrane to remove large particles and bacteria. The OD value of the extract was determined by a multifunctional enzyme marker, and the CSE obtained at an OD value of 0.25 at 405 nm was defined as 100% CSE stock solution. The CSE stock solution was readjusted to pH 7.40, and stored at 4°C for set aside. A pre-experiment was conducted on the treatment concentration and time of CSE. Cell viability gradually decreased with increasing CSE concentration. Further analysis of cell viability under 20% CSE showed that the cells were still proliferating at 24 h, 48 h and 72 h. It indicated that 20% CSE can be used to construct a COPD model of cells (Supplemental Figure 1A and B). CSE is prepared fresh before each assay and diluted to 20% as the working concentration. Importantly, the prepared CSE solution must be used in the assay within 30 min.

Cell Culture and Transfection

The human normal bronchial epithelial cell line (BEAS-2B), purchased from Pricella (Wuhan, China) and cultivated at 37°C under 5% CO_2. DMEM medium with 10% fetal bovine serum, penicillin and streptomycin was selected. After 24 h of culture, the cells in the CSE group were exposed to 20% CSE conditions for 24 h to simulate cells in COPD.

24 h before transfection, 1×10^5 cells were seeded into 6-well cell plates with 2 mL DMEM medium, and the degree of cell fusion was 60–70% at transfection. The desired small fragments such as miR-335-5p mimic/inhibitor or mimic/inhibitor NC were transfected into BEAS-2B cells with the aid of Lipofectamine 2000 (Invitrogen, USA). After 48 h of transfection, cells were gathered for subsequent experiments.

RT-qPCR

The required reagents including Trizol (Ambion, USA), chloroform, isopropanol, and 75% ethanol were pre-cooled in 4°C for extraction of total RNA from serum, peripheral blood mononuclear cells and BEAS-2B cells. RNA concentration was measured using a Nano Drop 2000 (Thermo, USA). Reverse transcription was performed according to the purchased PrimeScript RT Kit (Takara, Japan) and instructions. The reaction system is prepared according to the requirements, and the PCR reaction is set up on the machine. The $2^{-\Delta\Delta Ct}$ method was used for data statistics.

Cell Proliferation Assay

Cell proliferation was assayed by CCK8 (Dojindo, Japan). Cells in logarithmic growth phase with good status were taken from each group, inoculated in 96-well plates, and cultivated at 37°C in 5% CO_2 incubator. The absorbance at 450 nm was measured at 0 h, 24 h, 48 h, and 72 h. Prior to each assay, cells were cultured for 2 h with 10 μL of CCK-8 reagent.

Cell Apoptosis Assay

Apoptosis was assayed by Annexin V-FITC/PI assay. Cells were gathered from each group and cleaned once by PBS, then added appropriate amount of tryptic digestive enzyme and incubated at room temperature. Added complete culture medium to cells and removed them to a new centrifuge tube, then centrifuged at 3000 rpm/min for 5 min and discarded the supernatant. Cells were resuspended in PBS, centrifuged again, discarding discarded, and resuspended with 500 μL

binding buffer. Finally, 5 μL Annexin V-FITC and propidium iodide staining solution were added and incubated for 20 min away from light. Results were analyzed using Flowjo software.

Determination of TNF-α and IL-6 and Oxidative Stress Indicators

Enzyme-linked immunosorbent assay (ELISA) was adopted to assay TNF-α and IL-6 levels. The cells were centrifuged at 2000 rpm/min for 10 min to extract impurities and debris, and the supernatant was extracted. The assay was carried out based on instructions of the TNF-alpha ELISA Kit, Human (Sino Biological, China) and IL-6 ELISA Kit, Human (Sino Biological, China). The levels of oxidative stress indicators ROS, MDA and SOD were detected using kits (Beyotime, China).

Statistical Analysis

The experimental data were collected, and data were analyzed and plotted using GraphPad Prism 9.0 and SPSS 27.0 software. Results are shown as mean ± standard deviation (x ± s). Comparison among groups was achieved by *t*-test and one-way ANOVA. Diagnostic accuracy of miR-335-5p for COPD was determined by ROC curve. The associated risk factors for COPD were assessed using logistic regression analysis. The association of miR-335-5p with TNF-α and IL-6 was identified by Pearson correlation analysis. $P < 0.05$ is considered a statistically significant difference.

Results

Participants' Characteristics

A total of 117 COPD patients and 100 healthy participants were included in our research. The mean age was 64.27 ± 10.01 years and 65.26 ± 9.88 years respectively and their basic characteristics are shown in Table 1. The COPD group had 77 males and 40 females with a mean BMI of 22.98 ± 2.68 kg/m$^{2.}$ There were 58 males and 42 females in the healthy group with a mean BMI of 22.41 ± 2.48 kg/m^2. Participants in COPD and healthy groups were not statistically different in age ($P = 0.467$), gender ($P = 0.239$) and BMI ($P = 0.106$). However, smoking history ($P = 0.008$), FEV_1 ($P < 0.001$), FEV_1/FVC ($P < 0.001$), TNF-α ($P < 0.001$) and IL-6 ($P < 0.001$) showed major differences between COPD and healthy groups.

Table 1 Basic Characteristics of Participants

	Healthy (n=100)	COPD (n=117)	P-value
Age (years)	65.26±9.88	64.27±10.01	0.467
Gender (male/female)	58/42	77/40	0.239
BMI (kg/m^2)	22.41±2.48	22.98±2.68	0.106
Smoking (%)	31 (31.0%)	57 (48.7%)	0.008
FEV1 (predicted, %)	95.37±2.73	66.44±5.53	<0.001
FEV1/FVC (%)	81.97±1.40	60.49±2.54	<0.001
TNF-α (pg/mL)	9.53±1.51	21.26±5.10	<0.001
IL-6 (pg/mL)	6.82±2.02	13.11±3.15	<0.001
COPD grades			
I	–	22	–
II	–	78	–
III	–	17	–

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

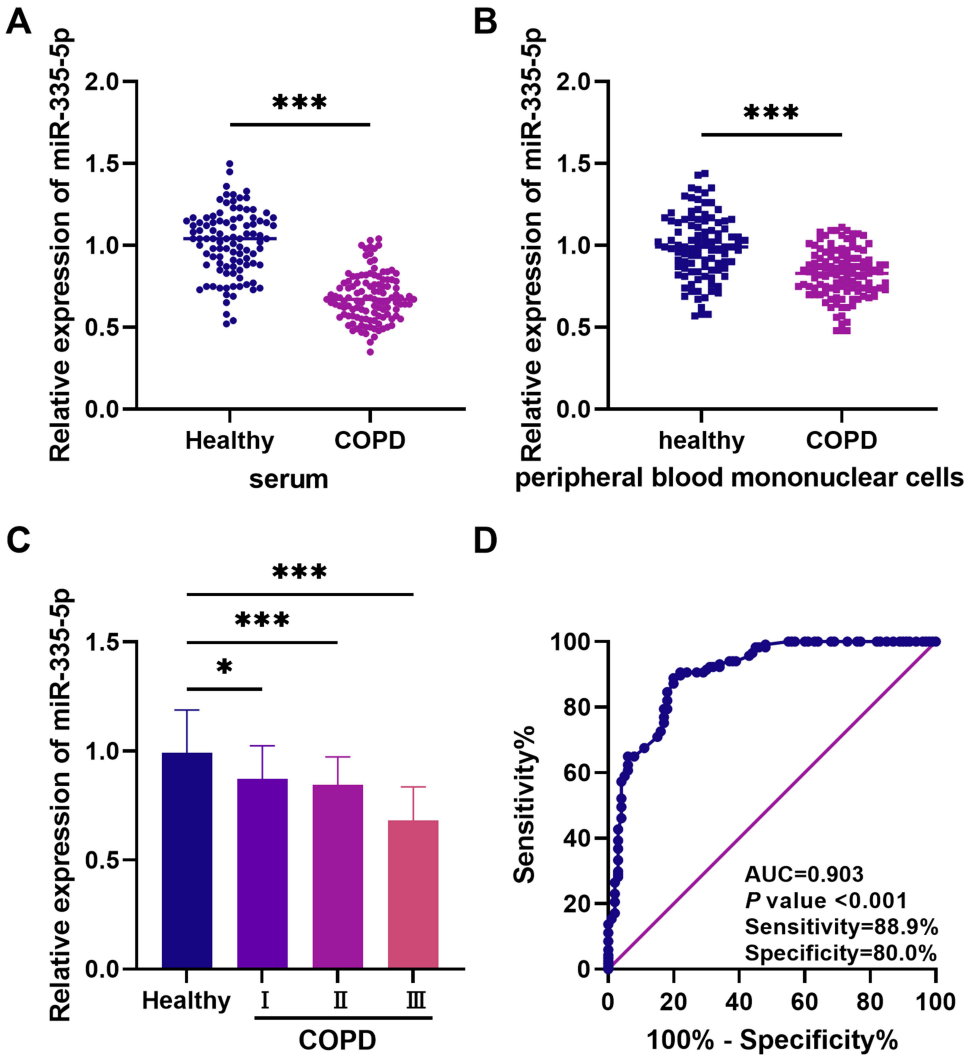

Figure 1 miR-335-5p expression in COPD. (**A**) miR-335-5p was downregulated in the serum of COPD patients. (**B**) miR-335-5p was downregulated in peripheral blood mononuclear cells of COPD patients. (**C**) With the grade of COPD patients increased, the level of miR-335-5p gradually decreased. (**D**) ROC curve showed that miR-335-5p distinguished between COPD patients and healthy individuals. *$P < 0.05$, ***$P < 0.001$.

miR-335-5p Was Downregulated in COPD Patients

miR-335-5p levels in serum and peripheral blood mononuclear cells were reduced in COPD patients compared to healthy controls (Figure 1A and B). In addition, as the grade of COPD patients increased, the level of miR-335-5p gradually decreased (Figure 1C). Serum miR-335-5p expression in healthy participants and COPD patients was subjected to ROC analysis, which showed that miR-335-5p had high sensitivity (88.9%) and specificity (80.0%) to distinguish COPD patients from healthy individuals, with a threshold value of 0.845 (AUC = 0.903, $P < 0.001$, Figure 1D). Logistic regression analysis demonstrated that miR-335-5p was a key risk factor for development of COPD ($P < 0.001$), and smoking history, FEV_1, FEV_1/FVC, TNF-α and IL-6 were also associated with COPD (Table 2). These findings suggested that miR-335-5p predicted COPD susceptibility.

miR-335-5p Negatively Correlated with Inflammatory Factors and Positively Correlated with FEV1 and FEV1/FVC in COPD Patients

To demonstrate the relationship of miR-335-5p with the COPD-related factors TNF-α,IL-6, FEV1, and FEV1/FVC, we adopted Pearson correlation analysis. The results indicated that miR-335-5p level was negatively correlated with TNF-α

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Table 2 Logistic Regression Analysis of Factors Associated with COPD

Parameters	OR	95% CI	P value
miR-335-5p	11.052	4.893–24.967	<0.001
Age (years)	1.604	0.720–3.576	0.248
Gender	1.815	0.788–4.180	0.162
BMI (kg/m^2)	1.777	0.816–3.871	0.148
Smoking (%)	2.591	1.132–5.930	0.024
FEV1 (predicted, %)	0.309	0.141–0.680	0.004
FEV1/FVC (%)	0.279	0.125–0.624	0.002
TNF-α (pg/mL)	2.886	1.324–6.293	0.008
IL-6 (pg/mL)	3.675	1.623–8.320	0.002

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

(r = −0.689, $P < 0.001$, Figure 2A) and IL-6 (r = −0.741, $P < 0.001$, Figure 2B) and positively correlated with FEV1 (r = 0.614, $P < 0.001$, Figure 2C) and FEV1/FVC (r = 0.526, $P < 0.001$, Figure 2D) in COPD patients. This suggested a correlation between miR-335-5p and clinical detection indicators in COPD patients.

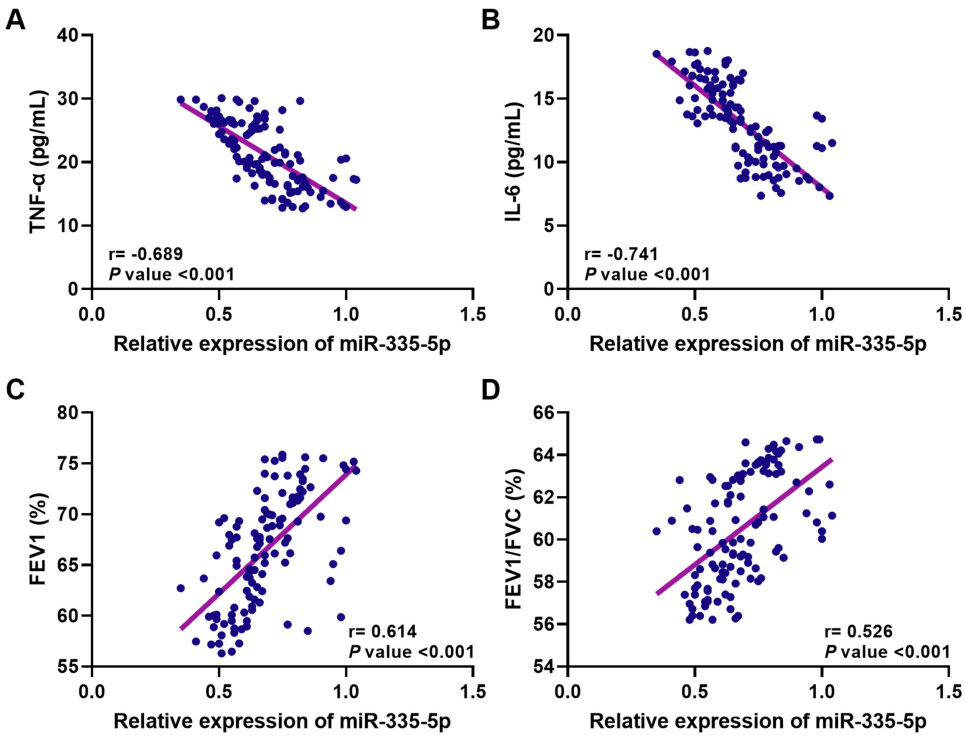

Figure 2 Relationship between miR-335-5p and inflammatory factors and pulmonary function indicators in COPD patients. (**A** and **B**) miR-335-5p was negatively correlated with TNF-α and IL-6. (**C** and **D**) miR-335-5p was positively correlated with FEV1 and FEV1/FVC.

Overexpression of miR-335-5p Attenuated CSE-Induced Cellular Injury

Validation of miR-335-5p in human bronchial epithelial cells by transfection with miR-335-5p mimic/inhibitor into BEAS-2B cells. RT-qPCR revealed that transfection of miR-335-5p mimic elevated miR-335-5p levels, while transfection of miR-335-5p inhibitor decreased miR-335-5p levels (Figure 3A). We treated BEAS-2B cells with 20% CSE to simulate cellular injury. As shown in Figure 3B and supplemental Figure 1C, miR-335-5p levels were reduced in both cells and supernatant after CSE treatment compared to control. In addition, transfection of miR-335-5p mimic in CSE-induced cells led to elevated miR-335-5p levels, and transfection of miR-335-5p inhibitor caused reduced miR-335-5p levels.

Cell proliferation assays showed that CSE treatment markedly reduced the proliferative capacity of BEAS-2B cells. Compared to CSE treatment, miR-335-5p upregulation led to enhanced cell proliferation, whereas miR-335-5p down-regulation decreased cell proliferation (Figure 3C). Apoptosis assay revealed that CSE-induced cells showed significantly higher levels of apoptosis. Overexpression and knockdown of miR-335-5p respectively inhibited and promoted the apoptotic compared to CSE-induced (Figure 3D). In addition, both TNF-α and IL-6 levels of BEAS-2B cells were elevated notably after CSE treatment. Transfection of miR-335-5p mimic dramatically decreased cells TNF-α and IL-6 levels, whereas transfection of miR-335-5p inhibitor elevated TNF-α and IL-6 levels (Figure 3E and F). We also examined the effect of CSE treatment on cellular oxidative stress. The results showed that CSE treatment increased the levels of ROS and MDA and decreased the level of SOD, while overexpression of miR-335-5p reversed this trend. (Figure 3G–I) These indicated that miR-335-5p upregulation attenuated CSE-induced cellular injury.

Discussion

In this study, we found that miR-335-5p was down-regulated in COPD. It was positively correlated with FEV1 and FEV1/FVC, and negatively correlated with IL-6 and TNF-α. CSE treatment of BEAS-2B cells resulted in decreased miR-335-5p levels, inhibited cell proliferation and SOD production, and elevated apoptosis, IL-6, TNF-α, ROS and MDA levels, while overexpression of miR-335-5p reversed this trend. These findings suggested that miR-335-5p up-regulation reduces COPD susceptibility and may serve as a potential target for COPD therapy.

With the rapid development of high-throughput technologies, multiple miRNAs have been found to be expressed differently between COPD patients and healthy individuals, thereby affecting the disease process.[19] For example, miR-223 level was upregulated in COPD patients and correlated with neutrophilic inflammation.[20] miR-378 affected COPD process by influencing cell proliferation, migration and apoptosis properties.[21] miR-335-5p expression was downregulated in lung fibroblasts from smokers and may affect functionality by targeting *Rb1, CARF* and *SGK3*, but the function in COPD has not yet been studied and discussed.[16] Here, we discovered that miR-335-5p was decreased in COPD patients' serum. Additionally, miR-335-5p exhibited high sensitivity and specificity in distinguishing between COPD and healthy individuals. This suggested that miR-335-3p has high accuracy in determining elevated COPD susceptibility. To further determine which factors are associated with COPD, logistic regression analysis showed that miR-335-5p was strongly correlated with elevated COPD susceptibility. This demonstrated that miR-335-5p may be a biomarker for diagnosis of COPD susceptibility.

The progression of COPD is closely related to abnormal inflammatory regulation, immune imbalance and oxidative stress.[22] It has been discovered that miRNAs are broadly implicated in the regulation of inflammation-related signaling pathways and modulate the development of diseases.[23] miR-155 may inhibit the NF-κB signaling pathway, which participates in the regulation of inflammation in bronchial asthma, allergic rhinitis, and other inflammatory conditions.[24] In cute exacerbation COPD patients, miR-126 expression upregulation was positively correlated with inflammatory factors of TNF-α, IL-1β, IL-6, and IL-17.[25] In the present work, miR-335-5p levels were revealed to be negatively correlated with TNF-α and IL-6. This suggested that miR-335-5p may participate in the advancement of COPD by regulating inflammatory factors. Zheng et al showed that in CSE-treated human bronchial epithelial cells, oxidative stress indicator MDA levels were increased and SOD levels were reduced.[26] This study also demonstrated similar results, and upregulation of miR-335-5p could reverse them. It is suggested that miR-335-5p may be involved in the progression of COPD through oxidative stress.

BEAS-2B is an immortalized human bronchial epithelial cell line with the functions and characteristics of normal human bronchial epithelial cells.[27] CSE alters basal cell differentiation and function and disrupts the epithelial cell

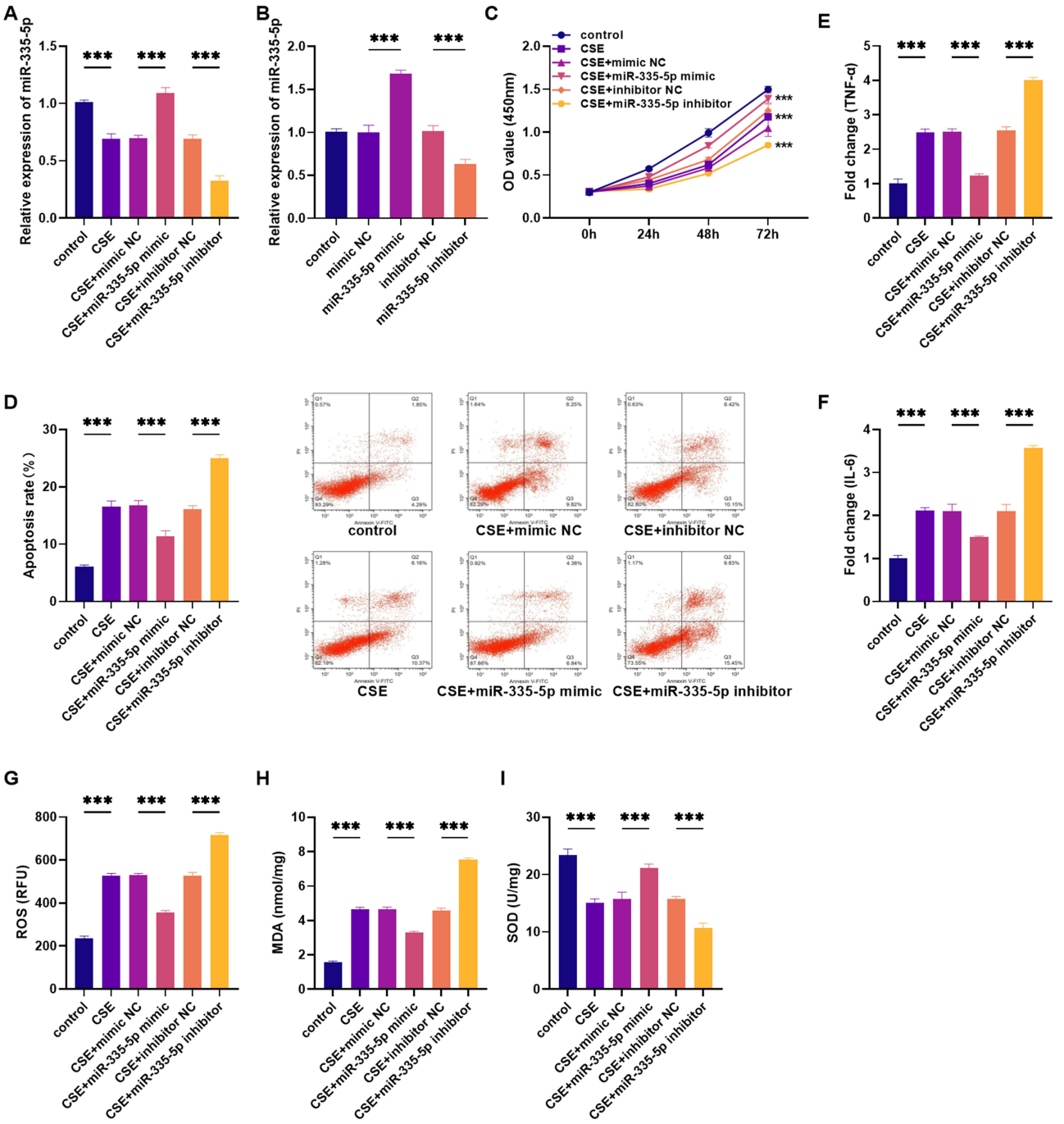

Figure 3 Effect of overexpression or knockdown of miR-335-5p on CSE-induced cellular injury. (**A**) Transfection of miR-335-3p mimic/inhibitor elevated or decreased miR-335-3p levels, respectively. (**B**) CSE-induced reduced miR-335-3p expression and transfection of miR-335-3p mimic/inhibitor elevated or reduced the effect of CSE on miR-335-5p expression in cells. (**C** and **D**) CSE treatment inhibited cell proliferation and promoted apoptosis, overexpression of miR-335-5p promoted cell proliferation and inhibited apoptosis, and knockdown of miR-335-5p showed the opposite trend. (**E** and **F**) CSE treatment elevated TNF-α and IL-6 levels and overexpression of miR-335-5p reduced them, whereas knockdown of miR-335-5p exhibited an opposite trend. (**G–I**) CSE treatment promoted ROS and MDA and inhibited SOD production, and miR-335-5p overexpression reversed this trend ***$P < 0.001$.

barrier.[28] In our research, CSE was applied to BEAS-2B cells to simulate cell injury. Liu's research showed that CSE treatment caused a significant increase in TNF-α and IL-6 in human bronchial epithelial cells and induced miR-937 levels decrease.[29] We found that CSE treatment reduced miR-335-5p levels, suppressed cell proliferation, facilitated apoptosis, and raised TNF-α, IL-6, ROS, and MDA levels, and reduced SOD levels. Overexpression of miR-335-5p reversed CSE-induced responses. This demonstrated that overexpression of miR-335-5p attenuated CSE-induced cell injury.

In addition, several studies have analyzed the mechanism and regulatory network of miRNAs in COPD. For example, Wu et al reported that miR-125a-5p drove lung epithelial cell senescence through inhibition of the Sp1/SIRT1 axis, increased HIF-1α acetylation, and thus promoted COPD progression.[30] miR-21 promoted COPD pathogenesis by regulating apoptosis and inflammation through the PTEN/Akt/NF-κB pathway.[31] Additionally, miR-1307-5p regulated COPD progression by targeting inhibition of FBXL16 and modulating HIF-1α stability.[32] In this study, it was found that miR-335-5p mediates the progression of COPD and may be involved in COPD by regulating the HIF-1α or NF-κB signaling pathways. However, its specific mechanism and downstream targets need further research.

Nevertheless, there are several limitations of the study. The sample size included in this study was small and concentrated in a single regional population, which may affect the universality of the conclusion. In the future, the sample size needs to be expanded to verify the predictive efficacy of miR-335-5p through multi-center. A knockout animal model of miR-335-5p is lacking to validate its dynamic role in COPD development. Subsequent studies are needed to construct an animal model of lung tissue-specific miR-335-5p knockout to clarify its role in different pathological stages of COPD. Furthermore, the regulatory mechanism by which miR-335-5p functions in COPD needs to be further explored.

Conclusion

In conclusion, reduced miR-335-5p promoted elevated COPD susceptibility and may be a biomarker for COPD diagnosis. Furthermore, overexpression of miR-335-5p alleviated CSE-induced cell injury, which indicated that miR-335-5p might be a potential therapeutic target for COPD.

Data Sharing Statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The research was approved by the Clinical Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University, and all participants signed an informed consent.

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Disclosure

The authors have no relevant financial or non-financial interests to disclose for this work.

References

1. Christenson SA, Smith BM, Bafadhel M, Putcha N. Chronic obstructive pulmonary disease. *Lancet*. 2022;399(10342):2227–2242. doi:10.1016/s0140-6736(22)00470-6
2. Halpin DMG, Criner GJ, Papi A, et al. Global initiative for the diagnosis, management, and prevention of chronic obstructive lung disease. The 2020 GOLD science committee report on COVID-19 and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2021;203(1):24–36. doi:10.1164/rccm.202009-3533SO
3. Bhatta L, Leivseth L, Mai XM, et al. GOLD classifications, COPD hospitalization, and all-cause mortality in chronic obstructive pulmonary disease: the HUNT study. *Int J Chronic Obstr*. 2020;15:225–233. doi:10.2147/copd.S228958
4. Corlateanu A, Mendez Y, Wang Y, Garnica RJA, Botnaru V, Siafakas N. Chronic obstructive pulmonary disease and phenotypes: a state-of-the-art. *Pulmonology*. 2020;26(2):95–100. doi:10.1016/j.pulmoe.2019.10.006
5. Wang C, Xu J, Yang L, et al. Prevalence and risk factors of chronic obstructive pulmonary disease in China (the China Pulmonary Health [CPH] study): a national cross-sectional study. *Lancet*. 2018;391(10131):1706–1717. doi:10.1016/s0140-6736(18)30841-9
6. Tan BWQ, Sim WL, Cheong JK, Kuan WS, Tran T, Lim HF. MicroRNAs in chronic airway diseases: clinical correlation and translational applications. *Pharmacol Res*. 2020;160:105045. doi:10.1016/j.phrs.2020.105045
7. Boateng E, Krauss-Etschmann S. miRNAs in lung development and diseases. *Int J Mol Sci*. 2020;21(8). doi:10.3390/ijms21082765
8. Skryabin GO, Beliaeva AA, Enikeev AD, Tchevkina EM. Extracellular vesicle miRNAs in diagnostics of gastric cancer. *Biochem Biokhimiia*. 2024;89(7):1211–1238. doi:10.1134/s0006297924070058

9. Petzuch B, Bénardeau A, Hofmeister L, et al. Urinary miRNA profiles in chronic kidney injury-benefits of extracellular vesicle enrichment and miRNAs as potential biomarkers for renal fibrosis, glomerular injury, and endothelial dysfunction. *Toxicological Sci*. 2022;187(1):35–50. doi:10.1093/toxsci/kfac028
10. Liu X, Cui H, Bai Q, Piao H, Song Y, Yan G. miR-128-3p alleviates airway inflammation in asthma by targeting SIX1 to regulate mitochondrial fission and fusion. *Int Immunopharmacol*. 2024;130:111703. doi:10.1016/j.intimp.2024.111703
11. Hayek H, Rehbini O, Kosmider B, et al. The regulation of fatty acid synthase by exosomal miR-143-5p and miR-342-5p in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2024;70(4):259–282. doi:10.1165/rcmb.2023-0232OC
12. Soofiyani SR, Hosseini K, Soleimanian A, et al. An overview on the role of miR-451 in lung cancer: diagnosis, therapy, and prognosis. *MicroRNA*. 2021;10(3):181–190. doi:10.2174/2211536610666210910130828
13. Wu JJ, Zhang PA, Chen MZ, et al. Analysis of key genes and miRNA-mRNA networks associated with glucocorticoids treatment in chronic obstructive pulmonary disease. *Int J Chronic Obstr*. 2024;19:589–605. doi:10.2147/copd.S441716
14. Kim RY, Sunkara KP, Bracke KR, et al. A microRNA-21-mediated SATB1/S100A9/NF-κB axis promotes chronic obstructive pulmonary disease pathogenesis. *Sci Trans Med*. 2021;13(621):eaav7223. doi:10.1126/scitranslmed.aav7223
15. Fu Y, Zhao J, Chen J, et al. miR-186-5p regulates the inflammatory response of chronic obstructive pulmonary disorder by targeting HIF-1α. *Mol Med Rep*. 2024;29(2). doi:10.3892/mmr.2024.13158
16. Ong J, van den Berg A, Faiz A, et al. Current smoking is associated with decreased expression of miR-335-5p in parenchymal lung fibroblasts. *Int J Mol Sci*. 2019;20(20). doi:10.3390/ijms20205176
17. Duecker RP, De Mir Messa I, Jerkic SP, et al. Epigenetic regulation of inflammation by microRNAs in post-infectious bronchiolitis obliterans. *Clin Transl Immunol*. 2022;11(2):e1376. doi:10.1002/cti2.1376
18. Sundar IK, Li D, Rahman I. Small RNA-sequence analysis of plasma-derived extracellular vesicle miRNAs in smokers and patients with chronic obstructive pulmonary disease as circulating biomarkers. *J Extracell Vesicles*. 2019;8(1):1684816. doi:10.1080/20013078.2019.1684816
19. Asensio VJ, Tomás A, Iglesias A, de Llano LP, Del Pozo V, Cosío BG. Eosinophilic COPD patients display a distinctive serum miRNA profile from asthma and non-eosinophilic COPD. *Archivos de bronconeumologia*. 2020;56(4):234–241. doi:10.1016/j.arbres.2019.09.020
20. Roffel MP, Maes T, Brandsma CA, et al. MiR-223 is increased in lungs of patients with COPD and modulates cigarette smoke-induced pulmonary inflammation. *Am J Physiol Lung Cell Mol Physiol*. 2021;321(6):L1091–l1104. doi:10.1152/ajplung.00252.2021
21. Qian G, Liao Q, Li G, Yin F. miR-378 associated with proliferation, migration and apoptosis properties in A549 cells and targeted NPNT in COPD. *PeerJ*. 2022;10(e14062). doi:10.7717/peerj.14062
22. Zhao X, Wu Y. Correlations of silent information regulator of transcription 1 (SIRT1) expression, inflammatory factors, and oxidative stress with pulmonary function in patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD). *Med Sci Monitor*. 2021;27(e929046). doi:10.12659/msm.929046
23. Ginckels P, Holvoet P. Oxidative stress and inflammation in cardiovascular diseases and cancer: role of non-coding RNAs. *Yale J Biol Med*. 2022;95(1):129–152.
24. Hu J, Huang S, Liu X, Zhang Y, Wei S, Hu X. miR-155: an important role in inflammation response. *J Immunol Res*. 2022;2022:7437281. doi:10.1155/2022/7437281
25. Wang C, Feng D, Dong S, He R, Fan B. Dysregulated circulating microRNA-126 in chronic obstructive pulmonary disease: linkage with acute exacerbation risk, severity degree, and inflammatory cytokines. *J Clin Lab Analysis*. 2022;36(3):e24204. doi:10.1002/jcla.24204
26. Zheng C, Zhang Y, Zhao Y, Duan Y, Mu Q, Wang X. Circ-OSBPL2 contributes to smoke-related chronic obstructive pulmonary disease by targeting miR-193a-5p/BRD4 axis. *Int J Chronic Obstr*. 2021;16:919–931. doi:10.2147/copd.S298465
27. Lee Y, Ryu YJ. Morphologically and karyotypically atypical cells of 'normal' human bronchial epithelial cell line (Beas-2B). *Ultrastructural Pathology*. 2023;47(6):470–477. doi:10.1080/01913123.2023.2262561
28. Chen Z, Wu H, Fan W, et al. Naringenin suppresses BEAS-2B-derived extracellular vesicular cargoes disorder caused by cigarette smoke extract thereby inhibiting M1 macrophage polarization. *Front Immunol*. 2022;13:930476. doi:10.3389/fimmu.2022.930476
29. Liu T. miR-937 serves as an inflammatory inhibitor in cigarette smoke extract-induced human bronchial epithelial cells by targeting IL1B and regulating TNF-α/IL-17 signaling pathway. *Tob Induc Dis*. 2021;19:55. doi:10.18332/tid/138227
30. Wu H, Ma H, Wang L, et al. Regulation of lung epithelial cell senescence in smoking-induced COPD/emphysema by microR-125a-5p via Sp1 mediation of SIRT1/HIF-1a. *Int J Bio Sci*. 2022;18(2):661–674. doi:10.7150/ijbs.65861
31. Sai X, Qin C, Zhang Z, Yu H, Bian T. A miRNA-21-mediated PTEN/Akt/NF-κB axis promotes chronic obstructive pulmonary disease pathogenesis. *Int J Chronic Obstr*. 2024;19:1141–1151. doi:10.2147/copd.S453593
32. Yao LP, Wang ZK, Jiang XQ, et al. MiR-1307-5p enhances fibroblast transdifferentiation to exacerbate chronic obstructive pulmonary disease through regulating FBXL16/HIF1α axis. *Respir Res*. 2024;25(1):376. doi:10.1186/s12931-024-03007-6