

Clinical Significance and Potential Mechanism of miR-532-5p/CXCL1 Axis in Chronic Obstructive Pulmonary Disease

(chronic obstructive pulmonary disease / miR-532-5p / CXCL1 / inflammation / oxidative stress / biomarker)

YONGQUAN WANG, GUANGMING XIANG

Department of Respiratory and Critical Care Medicine, The People's Hospital of Yichang City, Yichang 443000, China

Abstract. Chronic obstructive pulmonary disease (COPD) involves persistent airflow limitation and chronic inflammation. To define the clinical and mechanistic role of the miR-532-5p/CXCL1 axis in COPD, this study used bioinformatic analysis of the GEO dataset GSE70080 to identify miR-532-5p, which was significantly down-regulated in COPD. Multiple databases predicted *CXCL1* as its potential target gene. The clinical study included 90 subjects (52 with COPD, 38 controls). We detected serum levels of miR-532-5p and *CXCL1* mRNA using RT-qPCR and evaluated their diagnostic value through ROC curves and logistic regression. Inflammatory factors (TNF- α , IL-1 β) and oxidative stress indicators (SOD, MDA) were measured by ELISA and colorimetry. A dual-luciferase reporter gene assay verified that miR-532-5p directly targets *CXCL1*. In BEAS-2B cells treated with cigarette smoke extract (CSE), we conducted functional recovery experiments by transfecting miR-532-5p mimics and *CXCL1* over-expression vectors to explore their regulatory role in cell injury. The study found that the expression of miR-532-5p in the serum of

COPD patients was down-regulated, and its level was correlated with the decline of lung function and the enhancement of inflammation. It also had a diagnostic value for COPD (AUC = 0.8229) and was an independent protective factor. Mechanistically, *CXCL1* was confirmed to be a direct target of miR-532-5p; in the cell smoke injury model; miR-532-5p inhibited *CXCL1* to alleviate inflammation and oxidative stress. This study identifies miR-532-5p as a potential protective factor in COPD that acts by targeting *CXCL1* to mitigate inflammation and oxidative stress, suggesting its diagnostic and therapeutic relevance.

Introduction

Chronic obstructive pulmonary disease (COPD) imposes substantial health burdens globally. It is characterized by persistent respiratory symptoms and irreversible airflow limitation. As the third leading cause of death worldwide, COPD affects over 250 million people, with incidence rising particularly in low- and middle-income countries (Niu et al., 2024; Huang et al., 2025). The disease develops through several mechanisms: chronic airway inflammation triggered by inhaled irritants, oxidative stress from reactive oxygen species, and dysregulated tissue remodelling caused by protease-antiprotease imbalance. Together, these processes lead to progressive airflow limitation and structural lung destruction (Parajuli et al., 2025).

The clinical management of COPD currently centres on dual bronchodilation (LAMA+LABA), which, compared to monotherapy, reduces hospitalization and mortality (Huang et al., 2025). However, patient responses vary widely. This heterogeneity stems partly from persistent airway inflammation driven by neutrophil extracellular traps (NETs), which also cause steroid resistance by suppressing GR α and HDAC2 (Mani et al., 2025). These findings underscore the need for biomarkers that accurately reflect underlying molecular pathways.

MicroRNAs (miRNAs) have emerged as pivotal post-transcriptional regulators and promising biomarkers in

Received September 17, 2025. Accepted January 9, 2026.

Corresponding author: Guangming Xiang, Department of Respiratory and Critical Care Medicine, The People's Hospital of Yichang City, No. 183, Shengli First Road, Yichang 443000, China. Tel: (+86) 071 764 861 67; E-mail: YC_Xianggm1972@163.com

Abbreviations: AUC – area under the curve, BMI – body mass index, COPD – chronic obstructive pulmonary disease, CSE – cigarette smoke extract, HCG – healthy controls, Hs-CRP – high-sensitivity C-reactive protein, LDH – lactate dehydrogenase, MDA – malondialdehyde, miRNAs – microRNAs, MUT – mutant, NC – negative control, NETs – neutrophil extracellular traps, OGD – oxygen-glucose deprivation, ROC – receiver operating characteristic, SOD – superoxide dismutase, WBC – white blood cell, WT – wild-type.

Copyright: © 2026 Author et al. This is an open access article published under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0).

chronic inflammatory diseases, capable of fine-tuning complex gene networks that control inflammation and tissue remodelling (Qiao et al., 2022; Kim and Lee, 2024). In COPD, several miRNAs actively contribute to disease progression. For example, miR-21 promotes neutrophil infiltration, while miR-320d deficiency exacerbates epithelial inflammation. Other miRNAs such as miR-34a, miR-613, and miR-377-3p have been implicated in vascular damage, epithelial-mesenchymal transition, and fibroblast senescence, respectively (Kim et al., 2021; Zhang et al., 2021; Lu et al., 2024; Fu et al., 2025; Huo et al., 2025). In contrast to these “disease-promoting” miRNAs, the potential involvement of miRNAs with intrinsic protective or regulatory functions in counteracting COPD progression is far less understood. Interestingly, miR-532-5p has been reported to exert protective roles in other respiratory disease contexts (Li et al., 2022), yet its role in COPD remains largely unexplored.

This study investigates how the miR-532-5p/CXCL1 regulatory axis contributes to COPD progression. It also evaluates its potential as both a diagnostic biomarker and a therapeutic target for early intervention.

Material and Methods

Bioinformatic analysis

To explore miRNA expression characteristics related to COPD, this study analysed dataset GSE70080 from the NCBI GEO database. The dataset included 16 healthy individuals and 16 individuals with COPD. We screened for miRNAs with significant differential expression using the following criteria: $|\log_2FC| > 1.5$ and $P < 0.05$.

Research design and ethical approval

This study included 90 subjects: 52 COPD patients and 38 healthy controls (HCG). All COPD patients were recruited from the respiratory outpatient department of Yichang People’s Hospital. The inclusion criteria for COPD patients were based on the “GOLD 2023 Guidelines” (Agustí et al., 2023) and included: (1) a post-bronchodilator $FEV_1/FVC < 0.7$; (2) the presence of persistent respiratory symptoms such as chronic cough, sputum production, and/or dyspnoea; and (3) clinical stability, defined as no acute exacerbations within the preceding 4 weeks while on a stable maintenance medication regimen. To ensure study accuracy and specificity, we excluded patients with other respiratory diseases (such as asthma or pulmonary fibrosis), malignant tumours, or recent infections. The 38 healthy controls were required to have normal lung function ($FEV_1/FVC > 0.7$). Strict screening through detailed inquiries and pulmonary function tests ensured that this group could serve as reliable controls.

We collected 5 ml of venous blood from all subjects in pyrogen-free vacuum tubes (BD Vacutainer® SST™ II, Becton, Dickinson and Company, Franklin Lakes, NJ). After collection, samples were left at room temperature for 30 minutes to allow complete clotting. Then, they

were transferred to a 4 °C environment and centrifuged at $1500 \times g$ for 15 min to separate the serum (Centrifuge 5424R, Eppendorf, Hamburg, Germany). After centrifugation, we carefully drew the supernatant serum and aliquoted it into sterile centrifuge tubes, each containing 200 μ l. To ensure sample quality, serum samples were immediately frozen at -80 °C. We strictly limited freeze-thaw cycles to no more than three to avoid damage to biomolecules and ensure the accuracy and reliability of subsequent experimental results. The study protocol was approved by the Institutional Ethics Committee at The People’s Hospital of Yichang City, and all participants provided written informed consent before enrolment.

RT-qPCR quantitative analysis of miR-532-5p and CXCL1 mRNA

We quantified the relative levels of miR-532-5p and CXCL1 mRNA in the serum and cell lysates stored at -80 °C using RT-qPCR. Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA). For miR-532-5p, cDNA synthesis was performed using the TaqMan MicroRNA Reverse Transcription Kit (Cat#4366596, Thermo, Waltham, MA) with specific primers (Assay ID: 002198). For CXCL1 mRNA, cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit (Thermo, Cat#4368814). Quantitative PCR analysis was performed in the QuantStudio 5 system with TaqMan Universal Master Mix II (Thermo, Cat#4440040,) in a final reaction volume of 20 μ l, containing 2 μ l of cDNA template. The thermal cycling conditions consisted of an initial step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. CXCL1 mRNA was detected using the TaqMan probe Hs00174577_m1. Using *U6* and *GAPDH* as internal reference genes, the expression levels of miR-532-5p and CXCL1 mRNA were standardized. Relative quantification was calculated via the $2^{-\Delta\Delta Ct}$ method.

Detection of CXCL1, inflammatory factors and oxidative stress indicators

Inflammatory factors tumour necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β) and superoxide dismutase (SOD) activities were detected using commercial human ELISA kits (Shanghai Enzyme-Linked Biotechnology, Shanghai, China, Cat#ml077385, Cat#ml058059 and Cat#ml063052, respectively). CXCL1 protein secretion levels were measured using a commercial human CXCL1 ELISA kit (FineTest, Palm coast, FL, Cat#EH0005). The detection ranges for standard samples in each kit were: TNF- α : 15.62–1000 pg/ml; IL-1 β : 6.25–400 pg/ml; SOD: 0.5–40 U/ml; CXCL1: 15.625–1000 pg/ml. The limits of detection (LLOD) were: 7.81 pg/ml, 3.13 pg/ml, 0.16 U/ml, and 9.375pg/ml, respectively. According to the manufacturer’s validation data, the intra-assay and inter-assay coefficients of variation (CV) for all assays are less than 10 %. Serum samples were thawed at room temperature and centrifuged

at $3000 \times g$ for 10 min at 4 °C to remove precipitated material. For cell culture supernatants, media were collected after 48 h of incubation, centrifuged at $2000 \times g$ for 5 min at 4 °C to remove cellular debris, and the resulting supernatant was used for analysis. Antibody coating, blocking, sample/standard incubation, enzyme-labelled antibody incubation and TMB colour reaction were performed according to the kit instructions. The reaction was terminated with 2M H₂SO₄, and absorbance was read at 450 nm using the BioTek Synergy H1 microplate reader.

The malondialdehyde (MDA) content was determined using the TBA colorimetric kit (Beijing Solaibao Technology, Beijing, China, Cat#BC0025). The sample was mixed with TBA reagent at a 1 : 3 volume ratio and incubated in a water bath at 100 °C for 15 min. The mixture was then cooled in an ice bath and centrifuged at $10,000 \times g$ for 10 min at 4 °C. The supernatant was collected, and absorbance was measured at 532 nm using a Mindray BS-600 fully automatic biochemical analyser.

Target gene prediction

To predict the target genes of miR-532-5p, we used multiple bioinformatic databases. First, we employed the miRDB database with a Target Score threshold greater than 90 to identify potential target genes that may bind to miR-532-5p. Next, we used the TargetScan v7.0 database to select genes containing conserved binding sites with a Cumulative weighted context++ score and Total context++ score less than -0.4 as candidate target genes. We also searched the GeneCards database using the keyword “COPD” and selected genes with a relevance score (GIFT value) exceeding 55 to identify those associated with COPD. Finally, we used the Venny 2.1 intersection tool to identify common target genes across all three databases. These overlapping genes were considered the most likely to be regulated by miR-532-5p.

Cell culture and transfection

Human bronchial epithelial cells (BEAS-2B, ATCC® CRL-9609™) were cultured in DMEM/F12 medium supplemented with 10 % FBS at 37 °C with 5 % CO₂. Cells were seeded in 6-well plates at 2.5×10^5 cells/well and incubated for 24 h to reach 80 % confluence. Transient co-transfection was performed using Lipofectamine 3000 (Thermo Fisher, L3000015) according to the manufacturer’s protocol. Plasmid constructs and miRNA mimics were diluted in Opti-MEM medium, mixed with the transfection reagent and added to each well. After 6 h, the medium was replaced with fresh complete DMEM/F12, and cells were cultured for an additional 48 h before functional analysis.

Dual-luciferase reporter gene assay

To confirm that miR-532-5p directly targets *CXCL1*, we commissioned Beijing Zeping Biotechnology Co., Ltd. (Beijing, China) to generate luciferase reporter vectors containing either the wild-type (WT) *CXCL1* 3’UTR

sequence or a mutant (MUT) version with a disrupted miR-532-5p binding site. BEAS-2B cells were co-transfected with either the WT or MUT vector along with miR-532-5p mimics or negative control mimics (NC). After 48 h, cellular lysates were prepared, and luciferase activity was measured using a dual-luciferase assay system.

Standardized preparation of cigarette smoke extract

This experiment used a cigarette smoke extract (CSE) model and functional rescue experiments to explore the role of miR-532-5p and *CXCL1* in CSE-induced BEAS-2B injury. The experimental process, adapted from the literature (Li et al., 2024), is as follows: first, CSE was prepared using a standardized protocol. Marlboro Red cigarettes (10 mg tar, 0.8 mg nicotine) were selected. Three cigarettes were burned, and the smoke was introduced into 10 ml serum-free DMEM at a flow rate of 1 l/min. The solution was then sterilized with a 0.22 µm filter membrane (Millipore, Burlington, MA, SLGP033RB) and pH was adjusted to 7.4. This solution was defined as 100 % CSE – representing the complete extraction from the specified number of cigarettes per unit volume of medium – and served as a standardized stock solution for subsequent dilution.

To ensure batch consistency and verify biological activity, the optical density (OD) of each batch was measured at 320 nm. The cytotoxic effect on BEAS-2B cells was assessed via a cell viability assay. Only batches with OD values and half-maximal inhibitory concentration (IC₅₀) within a 15 % coefficient of variation were used for experiments. The 100 % CSE solution was aliquoted and stored at -80 °C for no more than two weeks. For all cellular treatments, fresh working dilutions were prepared from the stock immediately before each experiment to minimize degradation of active components.

Statistical analysis

Statistical analyses were performed using SPSS 26.0 for clinical data processing and GraphPad Prism 9.4.1 for graphical presentation. All datasets underwent Shapiro-Wilk normality assessment. For two-group comparisons, we used an independent samples *t*-test (homogeneous variance) or Mann-Whitney U test (heterogeneous variance). For multi-group comparisons, we applied one-way ANOVA with Tukey post hoc testing (homogeneous variance) or Kruskal-Wallis with Dunn’s test (heterogeneous variance). Correlations were analysed using Pearson (normal distribution) or Spearman (non-normal distribution) methods. Diagnostic performance was evaluated through receiver operating characteristic (ROC) curve analysis, reporting area under the curve (AUC) with 95% confidence intervals. Multivariate assessment used binary logistic regression. Statistical significance was set at $P < 0.05$.

Results

Screening of miR-532-5p

We analysed the GSE70080 database and screened for differential miRNAs, including miR-532-5p, which showed significantly down-regulated expression in the COPD group (Fig. 1A, $\log_2FC = -1.80$, $P < 0.05$). After identifying miR-532-5p as a candidate molecule, we verified its clinical significance.

Clinical expression verification and protective effect of miR-532-5p

This study included two groups of subjects: HCG patients and COPD patients. All COPD patients presented with characteristic respiratory symptoms, including chronic cough, sputum production, and/or dyspnoea. Based on the GOLD 2023 criteria, the cohort comprised 43 patients (82.7 %) with moderate (stage II) and 9 patients (17.3 %) with severe (stage III) disease. As shown in Table 1, the groups showed no significant differences

in age, gender distribution, body mass index (BMI), or smoking history, indicating well-matched baseline characteristics. However, COPD patients exhibited significantly reduced pulmonary function metrics (including FEV₁ % predicted and FEV₁/FVC %) and markedly elevated inflammatory markers – C-reactive protein (Hs-CRP) and white blood cell (WBC) count. These findings confirm baseline comparability between groups while demonstrating expected disease-specific differences in pulmonary function and inflammation levels.

Serum RT-qPCR detection revealed that miR-532-5p expression in COPD patients was significantly lower than in healthy controls (Fig. 1B), suggesting it may be a protective molecule in COPD. The markedly decreased miR-532-5p level exhibited a strong and significant correlation with the substantially reduced lung function indicator, FEV₁ % Predict (Fig. 1C, $r = 0.714$, $P < 0.001$) and FEV₁/FVC % ratio (Fig. 1D, $r = 0.635$, $P < 0.001$). Additionally, miR-532-5p expression was negatively correlated with Hs-CRP (Fig. 1E, $r = -0.629$, $P < 0.001$) and WBC (Fig. 1F, $r = -0.576$, $P < 0.001$), suggesting its role in regulating inflammatory pathways.

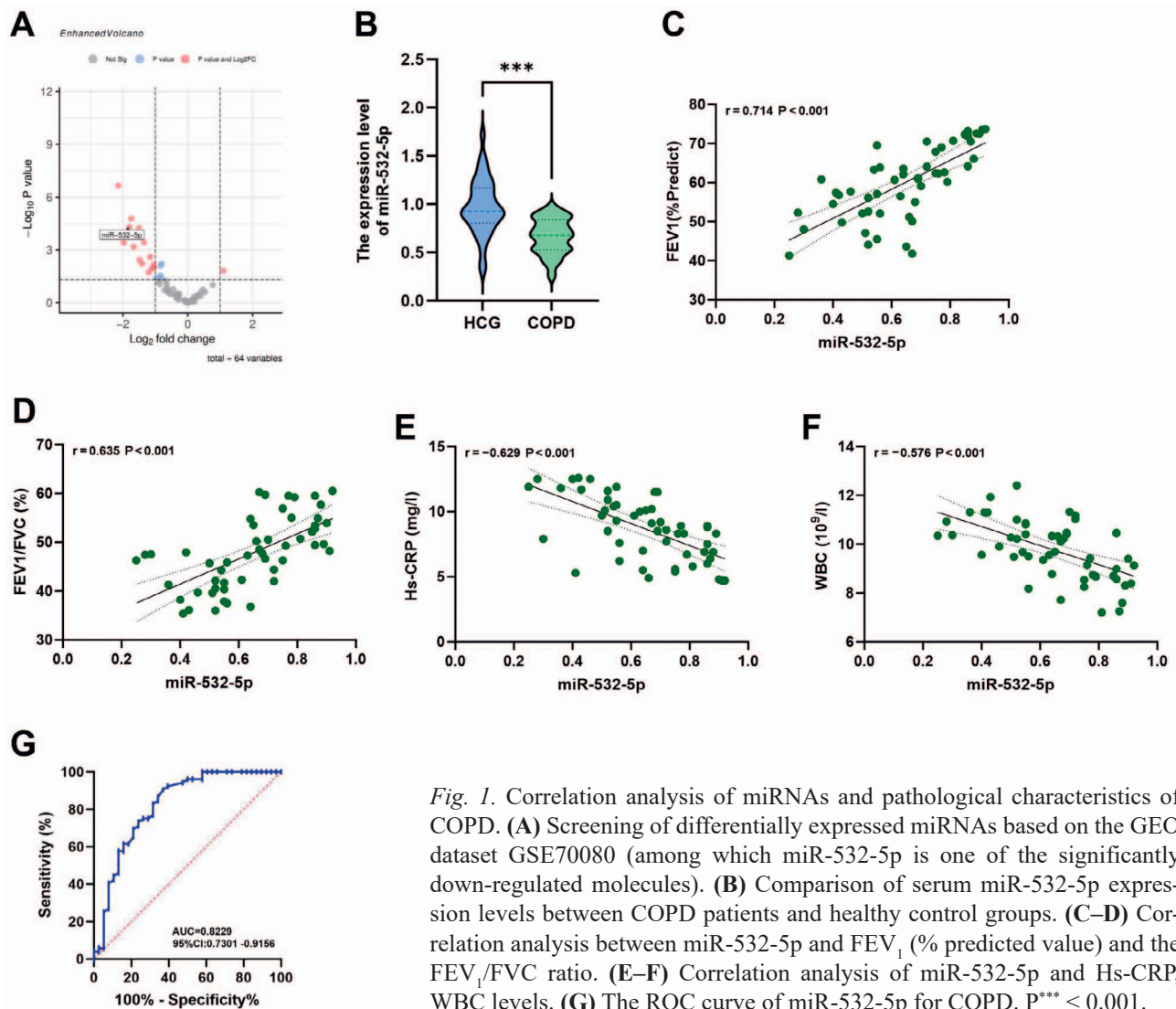


Fig. 1. Correlation analysis of miRNAs and pathological characteristics of COPD. (A) Screening of differentially expressed miRNAs based on the GEO dataset GSE70080 (among which miR-532-5p is one of the significantly down-regulated molecules). (B) Comparison of serum miR-532-5p expression levels between COPD patients and healthy control groups. (C–D) Correlation analysis between miR-532-5p and FEV₁ (% predicted value) and the FEV₁/FVC ratio. (E–F) Correlation analysis of miR-532-5p and Hs-CRP, WBC levels. (G) The ROC curve of miR-532-5p for COPD. $P^{***} < 0.001$.

Table 1. Comparison of baseline characteristics of COPD patients with HCG

Characteristic	Total	HCG	COPD	P value
Quantity	90	38	52	-
Age (years)	65.28 ± 4.89	64.03 ± 4.69	65.73 ± 5.00	0.236
Gender, Male (N%)	58.97 %	55.26 %	59.62 %	0.684
BMI (kg/m ²)	25.86 ± 3.1	26.30 ± 2.38	25.7 ± 2.7	0.353
Smoking history	37.78 %	28.95 %	44.23 %	0.143
FEV ₁ (%Predict)	68.60 ± 15.96	82.26 ± 5.74	60.01 ± 9.86	< 0.001
FEV ₁ /FVC (%)	56.63 ± 14.97	74.71 ± 5.13	47.78 ± 7.23	< 0.001
Hs-CRP (mg/l)	5.62 ± 4.00	1.43 ± 0.53	8.68 ± 2.39	< 0.001
WBC (10 ⁹ /l)	7.96 ± 2.46	5.49 ± 1.45	9.75 ± 1.2	< 0.001
GOLD stage, N (%)	-	-	-	-
I	-	-	0 (0)	-
II	-	-	43 (82.7)	-
III	-	-	9 (17.3)	-
IV	-	-	0 (0)	-

Abbreviations: HCG – healthy control group, COPD – chronic obstructive pulmonary disease, BMI – body mass index, FEV₁ – forced expiratory volume in 1 second, FVC – forced vital capacity, Hs-CRP – high-sensitivity C-reactive protein, WBC – white blood cell count. Grade I (mild): FEV₁ ≥ 80 % predicted, Grade II (moderate): 50 % ≤ FEV₁ < 80 % predicted, Grade III (severe): 30 % ≤ FEV₁ < 50 % predicted, Grade IV (very severe): FEV₁ < 30 % predicted.

To account for the potential confounding influence of smoking status, we conducted a further comparison of miR-532-5p levels between smokers and non-smokers within the COPD patient cohort (Fig. S1). No statistically significant difference was observed ($P > 0.05$), suggesting that the down-regulation of miR-532-5p is associated with the disease state of COPD rather than the smoking behaviour itself.

The diagnostic value and independent protective effect of miR-532-5p

To evaluate the diagnostic potential of miR-532-5p for COPD, ROC analysis yielded an AUC of 0.8229 (Fig. 1G, 95% CI: 0.7301–0.9156), indicating high discriminatory accuracy. Multivariate logistic regression further confirmed that miR-532-5p was an independent protective factor for COPD (OR = 0.061; 95% CI: 0.020–0.187, $P < 0.001$), even after adjusting for age, gender, BMI and smoking history (Table 2).

CSE-induced BEAS-2B cell model

Fig. 2A shows that cell viability decreased significantly as CSE concentration increased from 2.5 % to 15 % over 24 hours. This demonstrates a concentration-dependent toxic effect of CSE on BEAS-2B cells. For subsequent experiments, we selected 7.5 % CSE – the lowest concentration that significantly reduced cell viability.

To determine the optimal CSE exposure time, we evaluated cell viability at different time points (0, 6, 12, 24 and 48 h) using a CSE concentration of 7.5 % (Fig. 2B). Cell viability gradually decreased as treatment time

increased, indicating that the CSE's toxic effect on BEAS-2B cells is time-dependent. Notably, 24 h exposure to CSE induced significant yet non-lethal cytotoxic effects ($P < 0.001$), with cell viability remaining above 80 %. This makes the 24 h exposure period ideal for observing significant toxic responses while preserving cell viability.

In conclusion, this study found that a CSE concentration of 7.5 % and a 24-hour exposure time are optimal for establishing a BEAS-2B cell smoke model. These findings provide an experimental foundation for future studies examining how CSE affects cell function and signalling pathways.

Identification and validation of miR-532-5p target gene CXCL1

We used bioinformatic analysis to predict COPD-related genes targeted by miR-532-5p across three data-

Table 2. Multivariable logistic regression results of COPD-related factors

Characteristic	OR	95% CI		P value
		Lower	Upper	
Age	1.091	0.95	1.254	0.217
Gender	1.745	0.557	5.47	0.34
BMI	1.013	0.82	1.251	0.905
Smoking history	1.815	0.573	5.747	0.311
miR-532-5p	0.061	0.02	0.187	< 0.001

Abbreviations: BMI – body mass index

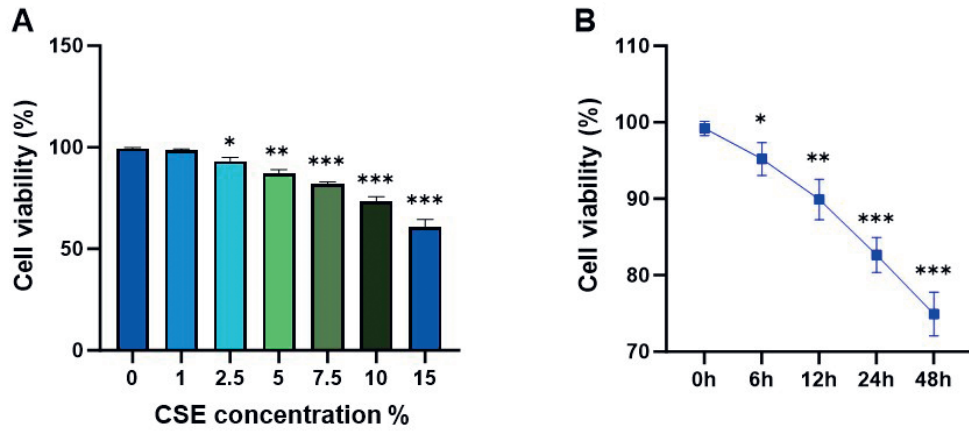


Fig. 2. The effect of CSE on the viability of BEAS-2B cells. (A) Effects of different concentrations of CSE on the viability of BEAS-2B cells. (B) The influence of CSE at different time points on the viability of BEAS-2B cells. P* < 0.05; P** < 0.01; P*** < 0.001.

bases: miRDB, TargetScan and GeneCards. By intersecting the results, we identified two common target genes: *CXCL1* and *KRAS* (Fig. 3A). Given that *CXCL1* plays a more established and central role in the CSE-induced inflammatory pathway and is more directly relevant to the phenotype under investigation in this study, we selected *CXCL1* for subsequent experimental validation. In addition, we detected the expression level of *CXCL1* in clinical serum samples and found that it was significantly up-regulated in COPD patients compared to HCG (Fig. 3B, P < 0.001).

To confirm that *CXCL1* is a direct functional target of miR-532-5p, we conducted a dual-luciferase reporter gene assay. The results (Fig. 3C) showed that co-transfection of the miR-532-5p mimic and the reporter vector containing the wild-type *CXCL1* 3'UTR sequence significantly reduced luciferase activity compared with the control group. However, when the *CXCL1* 3'UTR binding site was mutated in the reporter vector, the inhibitory effect of miR-532-5p was eliminated. Additionally, transfection of miR-532-5p mimics inhibited *CXCL1* expression (Fig. 3D). These results confirm that miR-532-5p

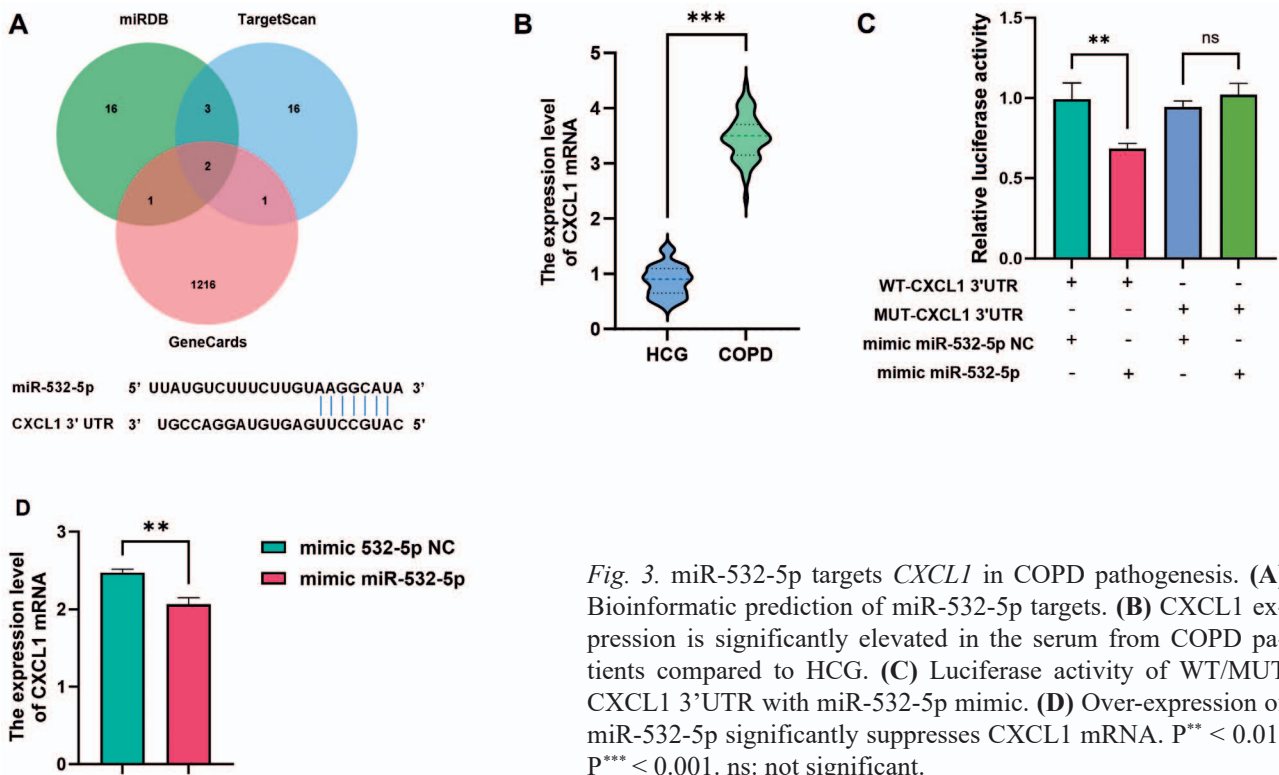


Fig. 3. miR-532-5p targets *CXCL1* in COPD pathogenesis. (A) Bioinformatic prediction of miR-532-5p targets. (B) *CXCL1* expression is significantly elevated in the serum from COPD patients compared to HCG. (C) Luciferase activity of WT/MUT *CXCL1* 3'UTR with miR-532-5p mimic. (D) Over-expression of miR-532-5p significantly suppresses *CXCL1* mRNA. P** < 0.01; P*** < 0.001. ns: not significant.

negatively regulates CXCL1 mRNA expression by specifically binding to its 3'UTR.

miR-532-5p alleviates CSE-induced inflammatory responses and oxidative stress by targeting and inhibiting CXCL1

More importantly, in the *in vitro* smoke exposure model constructed using CSE, we observed significant down-regulation of the expression level of miR-532-5p (Fig. 4A). Meanwhile, the expression level of CXCL1 mRNA in this model was consistently up-regulated (Fig. 4B). Consistent with this, the secretion of CXCL1 protein was also significantly increased upon CSE stimulation, as measured by ELISA. Transfection with the miR-532-5p mimic effectively suppressed this increase, while co-transfection with the *CXCL1* over-expression plasmid reversed the suppressive effect of the mimic (Fig. 4C).

CSE stimulation induced significant inflammatory and oxidative stress injuries. Compared with controls, CSE treatment significantly increased expression of inflammatory factors TNF- α and IL-1 β ($P < 0.001$), elevated oxidative stress marker MDA ($P < 0.001$), and decreased activity of antioxidant enzyme SOD ($P < 0.001$) (Fig. 4D–G). Transfection with the miR-532-5p mimic effectively alleviated these pathological changes. It inhibited TNF- α and IL-1 β up-regulation ($P < 0.01$ vs CSE group), reduced MDA accumulation ($P < 0.01$ vs CSE group) and partially restored SOD activity ($P < 0.05$ vs CSE group) (Fig. 4D–G). The CXCL1 rescue experiment provided key evidence. When miR-532-5p mimic and *CXCL1* over-expression were co-transfected (mimic + OE CXCL1 + CSE), the protective effect was substantially reversed. TNF- α , IL-1 β , and MDA levels in this group were significantly higher than in the mimic + CSE group ($P < 0.01$ for all comparisons), returning to levels not statistically different from the CSE-only group. Concurrently, the restored SOD activity was again suppressed compared to the mimic + CSE group ($P < 0.05$). These results demonstrate that miR-532-5p protects against CSE-induced injury by inhibiting CXCL1. Forced CXCL1 expression abolishes this protection, confirming *CXCL1* as a key functional downstream target of miR-532-5p.

Discussion

COPD is characterized by high morbidity and mortality rates, with its onset and progression closely associated with persistent airway inflammation and progressive airflow limitation (MacLeod et al., 2021). However, early diagnosis and disease monitoring lack sensitive and specific biomarkers, and current treatments have significant limitations. Identifying key regulatory molecules and understanding how they drive disease progression is essential for developing new diagnostic and therapeutic strategies. This study combined clinical sample analysis with *in vitro* functional validation to identify miR-532-5p as a candidate closely tied to the COPD's clinical features. The research revealed its critical role in driving inflam-

matory responses and oxidative stress through the regulation of specific target genes. This finding offers new insights into the molecular basis of COPD and establishes a foundation for future clinical applications.

This study confirmed in a clinically well-matched baseline cohort that COPD patients exhibit a significant decline in pulmonary function alongside systemic inflammation, consistent with the disease's classic pathological model of airflow limitation caused by chronic airway inflammation (Rajnoveanu et al., 2024). Although multiple studies have reported abnormal expression of other miRNAs in COPD (Huang et al., 2019; Yang et al., 2022), this study found that miR-532-5p, which is down-regulated in the COPD patient serum, shows significant correlations with both systemic inflammation severity and pulmonary function impairment. The significant positive correlation observed between miR-532-5p expression and key pulmonary function indicators (FEV₁ % predicted, $r = 0.714$; FEV₁/FVC ratio, $r = 0.635$) suggests its potential as a biomarker for assessing COPD disease severity. This finding aligns with the established consensus that circulating miRNA profiles correlate closely with the severity of airflow limitation (Zhuang et al., 2021). Additionally, this study found that miR-532-5p expression was significantly negatively correlated with systemic inflammatory markers (WBC and Hs-CRP). In COPD, elevated WBC levels typically reflect active recruitment of immune cells to the lungs, while increased Hs-CRP levels indicate the presence and severity of systemic inflammation (Chen et al., 2021). This finding suggests that miR-532-5p may participate in regulating inflammatory pathways. Its down-regulation may be a key factor driving the persistent inflammation characteristic of COPD. More importantly, miR-532-5p exhibits strong diagnostic ability for COPD (AUC = 0.8229) and serves as a protective factor independent of common risk factors. This finding supports the broader theory that circulating miRNAs can serve as valuable biomarkers for COPD (Cañas et al., 2020) by identifying a specific miRNA with significant diagnostic and protective relevance. Collectively, these findings suggest that miR-532-5p may not merely serve as a biomarker; its down-regulation likely plays a functional role in the inflammatory progression and lung function decline of COPD. This provides a clear clinical rationale for subsequent exploration of its specific molecular mechanisms.

To investigate the functional mechanisms of miR-532-5p in COPD at the cellular level, this study used human bronchial epithelial BEAS-2B cells and CSE to establish an *in vitro* model. Airway epithelium is a key target for cigarette smoke exposure (Park et al., 2015), and the BEAS-2B cell line is a well-established model for studying CSE-induced oxidative stress and inflammatory responses (Liu et al., 2024). This system provides an ideal foundation for subsequent research. To identify miR-532-5p target genes, we cross-analysed multiple prediction tools and selected the two highest-scoring candidates: *KRAS* and *CXCL1*. While *KRAS*, as a critical signalling node, may be implicated in COPD processes such as

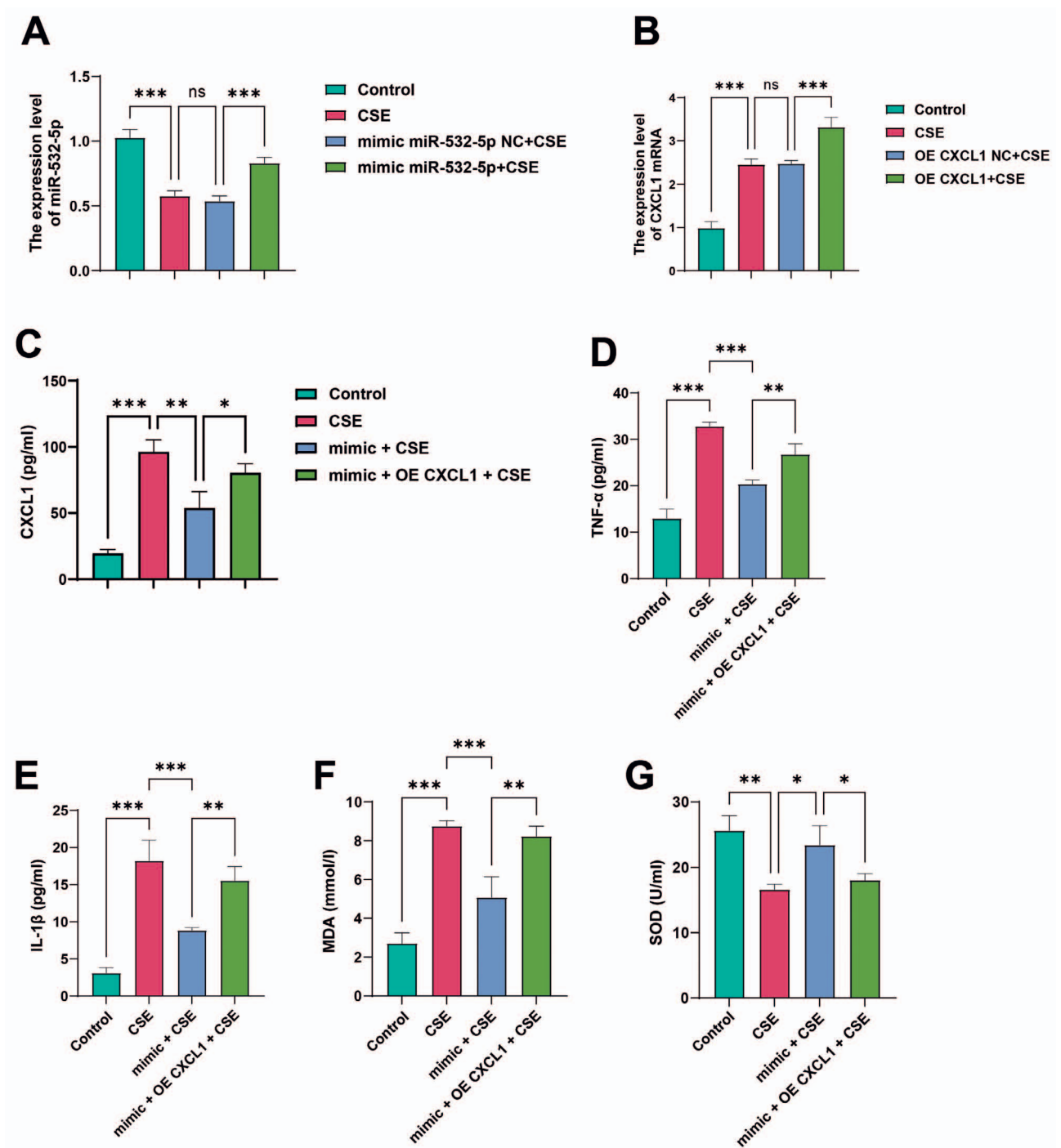


Fig. 4. miR-532-5p antagonizes CSE-induced inflammatory responses and oxidative stress by targeting *CXCL1*. **(A)** The expression level of miR-532-5p under CSE treatment and over-expression conditions. **(B)** The expression level of *CXCL1* mRNA under CSE treatment and over-expression conditions. **(C)** *CXCL1* concentration in cell culture supernatants was determined by ELISA. **(D–E)** The influence of miR-532-5p and *CXCL1* on the levels of inflammatory factors TNF- α and IL-1 β . **(F)** The influence of miR-532-5p and *CXCL1* on the MDA level. **(G)** The influence of miR-532-5p and *CXCL1* on the SOD level. $P^* < 0.05$; $P^{**} < 0.01$; $P^{***} < 0.001$. ns: not significant.

airway remodelling through functional abnormalities (Wei et al., 2025), *CXCL1* offers a more direct link to the COPD's core inflammatory phenotype. As a well-established neutrophil chemotactic factor (Zhou et al., 2023), *CXCL1* provides a robust evidence chain connect-

ing it to COPD inflammation. Given our preliminary findings linking miR-532-5p down-regulation to systemic inflammation, *CXCL1* investigation offers a direct pathway to elucidate the miR-532-5p's protective role through inflammatory regulation.

Subsequently, dual luciferase reporter assays confirmed that *CXCL1* is a direct target of miR-532-5p, providing molecular validation of this regulatory relationship. Furthermore, in a CSE-induced cellular injury model, this study demonstrated that miR-532-5p alleviates CSE-induced inflammation and oxidative stress by specifically inhibiting *CXCL1*; conversely, over-expression of *CXCL1* reverses the protective effects of miR-532-5p. This finding offers a novel perspective on understanding inflammatory regulation in COPD. Notably, the anti-inflammatory and antioxidant role of miR-532-5p has been observed in other pathological contexts as well. For instance, in femoral head necrosis, miR-532-5p mitigates oxidative stress and apoptosis in chondrocytes by targeting *ABLI* (Shang et al., 2024). Similarly, under oxygen-glucose deprivation conditions, miR-532-5p over-expression reduces lactate dehydrogenase release and ROS generation (Lu et al., 2021). These studies collectively highlight the consistent regulatory function of miR-532-5p across different disease models. Our findings reveal that miR-532-5p directly suppresses *CXCL1* expression at the RNA level. This suggests that during COPD pathogenesis, reduced levels of regulatory factors such as miR-532-5p may significantly contribute to inflammatory mediator over-expression (e.g., *CXCL1*), alongside activation of known inflammatory pathways. Crucially, *CXCL1* functions not merely as a terminal inflammatory output, but as a central signalling molecule capable of amplifying its own production and driving downstream pathology.

CXCL1 is elevated in COPD patients and models, a process driven by NF- κ B signalling (Inui et al., 2018). Upon binding to its receptor CXCR2, *CXCL1* activates key pro-inflammatory cascades, notably NF- κ B and MAPK (p38/JNK) pathways (Liu et al., 2025). This activation directly explains the observed up-regulation of cytokines (e.g., TNF- α , IL-1 β), elevated oxidative stress markers (e.g., MDA) and antioxidant depletion (e.g., SOD) in our model (Tourelakopoulos et al., 2025). Therefore, we propose that miR-532-5p serves as an upstream regulator within a self-amplifying inflammatory loop: its down-regulation by CSE elevates *CXCL1*, which activates NF- κ B/MAPK signalling via CXCR2, thereby reinforcing inflammation and oxidative stress, and perpetuating airway epithelial injury in COPD. By identifying miR-532-5p as a direct repressor of *CXCL1*, this study reveals a potential intervention point in the COPD's inflammatory cascade. Given *CXCL1*'s signalling through pathways such as NF- κ B, disrupting this axis may attenuate the inflammation-oxidative stress cycle in airway diseases.

This study provides evidence supporting the role of miR-532-5p in COPD pathogenesis, yet several limitations warrant acknowledgment. Clinically, our cohort primarily consisted of patients with moderate to severe (GOLD stage II–III) COPD. While this reflects a commonly encountered clinical population, it may limit the generalizability of our findings to patients with mild (GOLD I) or very severe (GOLD IV) disease. Mechanistically,

our conclusions are primarily based on an *in vitro* cigarette smoke extract model using bronchial epithelial cells, which cannot fully replicate the complex multicellular environment of COPD or confirm the physiological relevance of miR-532-5p modulation *in vivo*. Furthermore, while *CXCL1* was validated as a functional target, contributions from other predicted targets and the proposed downstream NF- κ B/MAPK pathways require further investigation. These limitations highlight the preliminary nature of our findings. Future studies involving more stratified clinical cohorts, advanced multicellular or animal models and direct pathway validation will be essential to advance this axis toward therapeutic application.

In conclusion, this study systematically clarified the molecular mechanism by which miR-532-5p exerts anti-inflammatory and antioxidant protective effects in COPD by targeting and inhibiting *CXCL1*, providing a new direction for the diagnosis and treatment of COPD.

Conflict of interest

Authors declare no conflict of interest.

References

- Agustí, A., Celli, B. R., Criner, G. J. et al. (2023) Global initiative for chronic obstructive lung disease 2023 report: GOLD executive summary. *Eur. Respir. J.* **61**, 2300239.
- Cañas, J. A., Rodrigo-Muñoz, J. M., Sastre, B. et al. (2020) MicroRNAs as potential regulators of immune response networks in asthma and chronic obstructive pulmonary disease. *Front. Immunol.* **11**, 608666.
- Chen, J., Yang, Z., Yuan, Q. et al. (2021) Prediction of gold stage in patients hospitalized with COPD exacerbations using blood neutrophils and demographic parameters as risk factors. *BMC Pulm. Med.* **21**, 329.
- Fu, H., Liu, K., Zheng, Y. et al. (2025) Upregulation of ARHGAP18 by miR-613 inhibits cigarette smoke extract-induced apoptosis and epithelial-mesenchymal transition in bronchial epithelial cells. *Int. J. Chron. Obstruct. Pulmon. Dis.* **20**, 2525-2537.
- Huang, J., Jiang, W., Tong, X. et al. (2019) Identification of gene and microRNA changes in response to smoking in human airway epithelium by bioinformatics analyses. *Medicine (Baltimore)* **98**, e17267.
- Huang, Y. J., Wang, K. Y., Chien, W. C. et al. (2025) Paradigm shift in the treatment of chronic obstructive pulmonary disease improves patient outcomes. *Int. J. Chron. Obstruct. Pulmon. Dis.* **20**, 1965-1972.
- Huo, M., Mei, H., Zhang, Y. et al. (2025) Expression and regulatory mechanism of miR-34a in neonatal rat model of bronchopulmonary dysplasia induced by hyperoxia. *Beijing Da Xue Xue Bao Yi Xue Ban* **57**, 237-244. (in Chinese)
- Inui, T., Watanabe, M., Nakamoto, K. et al. (2018) Bronchial epithelial cells produce *CXCL1* in response to LPS and TNF α : a potential role in the pathogenesis of COPD. *Exp. Lung Res.* **44**, 323-331.
- Kim, M. E., Lee, J. S. (2024) Mechanisms and emerging regulators of neuroinflammation: exploring new therapeutic strategies.

- tegies for neurological disorders. *Curr. Issues Mol. Biol.* **47**, 8.
- Kim, R. Y., Sunkara, K. P., Bracke, K. R. et al. (2021) A microRNA-21-mediated SATB1/S100A9/NF- κ B axis promotes chronic obstructive pulmonary disease pathogenesis. *Sci. Transl. Med.* **13**, eaav7223.
- Li, S., Zhu, C., Wang, Q. et al. (2022) miR-532-5p inhibits non-small cell lung cancer progression in vivo and in vitro by targeting yin yang 1. *Crit. Rev. Eukaryot. Gene Expr.* **32**, 47-61.
- Li, X., Jiang, X., Zeng, R. et al. (2024) Formononetin attenuates cigarette smoke-induced COPD in mice by suppressing inflammation, endoplasmic reticulum stress, and apoptosis in bronchial epithelial cells via AhR/CYP1A1 and AKT/mTOR signaling pathways. *Phytother. Res.* **38**, 1278-1293.
- Liu, C., Zhu, X., Aga, E. et al. (2024) Ebeiedinone and peimisine inhibit cigarette smoke extract-induced oxidative stress injury and apoptosis in BEAS-2B cells. *Cell Stress Chaperones* **29**, 697-708.
- Liu, T., Zhang, X., Chen, R. et al. (2025) Uncovering common disease mechanisms and critical biomarkers in Crohn's disease with concurrent psoriasis and exploring potential therapeutic agents. *PLoS One*, **20**, e0324007.
- Lu, F., Yao, L. P., Gao, D. D. et al. (2024) MicroRNA-377-3p exacerbates chronic obstructive pulmonary disease through suppressing ZFP36L1 expression and inducing lung fibroblast senescence. *Respir. Res.* **25**, 67.
- Lu, Z., Li, L., Wei, L. et al. (2021) Long non-coding RNA LOC366613 alleviates the cerebral ischemic injury via regulating the miR-532-5p/phosphatase and tensin homolog axis. *Bioengineered* **12**, 2511-2522.
- MacLeod, M., Papi, A., Contoli, M. et al. (2021) Chronic obstructive pulmonary disease exacerbation fundamentals: diagnosis, treatment, prevention and disease impact. *Respirology* **26**, 532-551.
- Mani, S., Li, Z., Badji, H. et al. (2025) Dysfunction of COX-2/mPGES-1/PGE₂ pathway and EP4 receptor in bronchi from COPD patients. *Prostaglandins Leukot. Essent. Fatty Acids* **206**, 102685.
- Niu, Y., Niu, H., Meng, X. et al. (2024) Associations between air pollution and the onset of acute exacerbations of COPD: a time-stratified case-crossover study in China. *Chest* **166**, 998-1009.
- Parajuli, N., Subedi, K., Solone, X. K. et al. (2025) Epigenetic control of alveolar macrophages: impact on lung health and disease. *Cells* **14**, 640.
- Park, Y. H., Kim, D., Dai, J. et al. (2015) Human bronchial epithelial BEAS-2B cells, an appropriate in vitro model to study heavy metals induced carcinogenesis. *Toxicol. Appl. Pharmacol.* **287**, 240-245.
- Qiao, X., Hou, G., He, Y. L. et al. (2022) The novel regulatory role of the lncRNA-miRNA-mRNA axis in chronic inflammatory airway diseases. *Front. Mol. Biosci.* **9**, 927549.
- Rajnoveanu, R. M., Harangus, A., Todea, D. A. et al. (2024) Opioids in treatment of refractory dyspnea in chronic obstructive pulmonary disease: yes, no or maybe. *J. Pers. Med.* **14**, 318.
- Shang, P., Liu, Y., Ren, J. et al. (2024) Overexpression of miR-532-5p restrains oxidative stress response of chondrocytes in nontraumatic osteonecrosis of the femoral head by inhibiting ABL1. *Open Med. (Wars.)* **19**, 20240943.
- Tourlakopoulos, K., Mavrovounis, G., Kontopoulou, L. et al. (2025) Unmasking convergent inflammatory and oxidative pathways in asthma. *Allergy* **80**, 1801-1806.
- Wei, C., Zhu, Y., Chen, C. et al. (2025) Analysis of immune characteristics and inflammatory mechanisms in COPD patients: a multi-layered study combining bulk and single-cell transcriptome analysis and machine learning. *Front. Med. (Lausanne)* **12**, 1592802.
- Yang, T., Wang, J., Zhao, J. et al. (2022) Current and prospective applications of exosomal microRNAs in pulmonary fibrosis (Review). *Int. J. Mol. Med.* **49**, 37.
- Zhang, Y., Wang, L., Mutlu, G. M. et al. (2021) More to explore: further definition of risk factors for COPD – differential gender difference, modest elevation in PM_{2.5}, and e-cigarette use. *Front. Physiol.* **12**, 669152.
- Zhou, C., Gao, Y., Ding, P. et al. (2023) The role of CXCL family members in different diseases. *Cell Death Discov.* **9**, 212.
- Zhuang, Y., Hobbs, B. D., Hersh, C. P. et al. (2021) Identifying miRNA-mRNA networks associated with COPD phenotypes. *Front. Genet.* **12**, 748356.