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RESEARCH ARTICLE

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The clinical value of lncRNA MALAT1 and its targets miR-125b, miR-133, miR-146a, and miR-203 for predicting disease progression in chronic obstructive pulmonary disease patients

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Abstract

Objective: The study aimed to explore the correlations of long non-coding RNA MALAT1 (lncRNA MALAT1) and its targets microRNA (miR)-125b, miR-133, miR-146a, and miR-203 with acute exacerbation risk, inflammation, and disease severity of chronic obstructive pulmonary disease (COPD).

Methods: Plasma samples were obtained from 120 acute exacerbation COPD (AECOPD) patients, 120 stable COPD patients, and 120 healthy controls (HCs). RT-qPCR was conducted to detect lncRNA MALAT1 expression and its target miRNAs, and ELISA was performed to detect the inflammatory cytokines.

Results: LncRNA MALAT1 was highest in AECOPD patients followed by stable COPD patients and then HCs, which distinguished AECOPD patients from HCs (AUC: 0.969, 95% CI: 0.951-0.987) and stable COPD patients (AUC: 0.846, 95% CI: 0.798-0.894). Furthermore, lncRNA MALAT1 positively correlated with GOLD stage in both AECOPD and stable COPD patients. Regarding inflammatory cytokines, lncRNA MALAT1 positively correlated with tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, IL-17, and IL-23 in both AECOPD and stable COPD patients. Besides, lncRNA MALAT1 negatively correlated with miR-125b, miR-146a, and miR-203 in AECOPD patients and reversely correlated with miR-125b and miR-146a in stable COPD patients. Notably, miR-125b, miR-133, miR-146a, and miR-203 were the lowest in AECOPD patients, followed by stable COPD patients, and then HCs; miR-125b, miR-133, miR-146a, and miR-203 negatively correlated with inflammation and GOLD stage in AECOPD and stable COPD patients.

Conclusion: LncRNA MALAT1 exhibits clinical implications in acute exacerbation risk prediction and management of COPD via the inner-correlation with its targets miR-125b, miR-146a, and miR-203.

KEYWORDS

chronic obstructive pulmonary disease, disease risk, disease severity, inflammation, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1, target microRNAs

Shuang Liu and Min Liu contributed equally to this work.

1 | INTRODUCTION

Long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (LncRNA MALAT1), a highly abundant and evolutionary conserved lncRNA, locates on chromosomal 11, which is essential in the regulation of various pathology-physiological processes such as inflammation and immunity.[1-4] For instance, lncRNA MALAT1 evokes and intensifies inflammation via activating p38 mitogen-activated protein kinase (MAPK)/nuclear factor kappa-light-chain enhancer of activated B-cell (NF-κB) signaling pathway in sepsis.[4] Another study reveals that lncRNA MALAT1 knockdown inhibits inflammatory responses in lipopolysaccharide (LPS)-induced acute lung injury.[2] Additionally, lncRNA MALAT1 promotes pro-inflammatory M1 alveolar macrophage activation and suppresses the alternative M2 alveolar macrophage and profibrotic activation, which is involved in the pulmonary inflammation and injury.[3] Clinically, lncRNA MALAT1 high expression exhibits the potential for predicting elevated acute respiratory distress syndrome risk in sepsis patients.[5]

Chronic obstructive pulmonary disease (COPD), a heterogenous disease, is defined by the persistent limitation of airflow that is not fully reversible, progressive deterioration of pulmonary function and increasing airway obstruction.[6,7] It is the fourth leading cause of death worldwide with over 3 million death annually.[8] Acute exacerbation of COPD (AECOPD) is frequent in patients with COPD, which deteriorates patients' symptoms such as dyspnea, cough, sputum production, and airflow obstruction.[8,9] Frequent exacerbations result in the deterioration of lung function, decreased physical activity, declined patients' quality of life, and increased risk of death.[10] Therefore, it is of a great need to explore potential biomarkers for the early and timely identification of acute exacerbation risk and disease management in COPD patients.

Known that COPD is a type of severe pulmonary inflammatory disease characterized by excessive inflammatory response, inappropriate immune activation, and lung injury, and that lncRNA MALAT1 contributes to the excessive inflammatory responses, aberrant macrophage activation, and injury of lung,[2-4,11] we speculated that lncRNA MALAT1 might be involved in the development and progression of COPD as well. Meanwhile, lncRNA MALAT1 accelerates inflammation via interacting with microRNA (miR)-125b, miR-133, miR-146a, or miR-203 in other inflammatory diseases (including sepsis, ischemia-reperfusion injury, acute lung injury, and myocardial-reperfusion injury) rather than COPD.[2,4,12-14] As an example, lncRNA MALAT1 sponges miR-133 to amplify inflammation in ischemia-reperfusion injury.[13] Another study illuminates that lncRNA MALAT1 is negatively associated with miR-125b in sepsis patients.[14] As for COPD, relevant report is still lacking. Therefore, this study aimed to explore the correlations of lncRNA MALAT1 and its targets miR-125b, miR-133, miR-146a, and miR-203 with acute exacerbation risk, inflammation, and disease severity of COPD.

2 | MATERIALS AND METHODS

2.1 | Participants

Between July 2017 and June 2019, 120 AECOPD patients and 120 stable COPD patients were consecutively recruited from our hospital. All patients were diagnosed as COPD according to the criteria of Global Initiative for Chronic Obstructive Lung Disease (GOLD)[15] and were more than 40 years old. The AECOPD were defined as the COPD patients with at least 2 of the following major symptoms (increased dyspnea, increased sputum purulence, increased sputum volume) or 1 major and 1 minor symptom (nasal discharge/congestion, wheeze, sore throat, cough) for at least 2 consecutive days. The stable COPD is defined as the COPD patients without medication changes or exacerbation in 3 months. The exclusion criteria for AECOPD patients and stable COPD patients were as follows: (1) complicated with asthma, pneumonia, or other relevant respiratory diseases; (2) history of hematological malignancies, solid tumors or autoimmune diseases; (3) seropositivity for human immunodeficiency virus (HIV); and (4) pregnant or lactating woman. It was to be noted that the identification and grouping of AECOPD patients and stable COPD patients was only based on the initial disease status after enrollment. And a same patient would not be enrolled in both AECOPD group and stable COPD group due to the changes in disease status. For example, a patient who was recruited in AECOPD group, he would not be enrolled in stable COPD group even his clinical condition changed to stable COPD, meanwhile, if a patient recruited in stable COPD group, he would not be enrolled in AECOPD group even his clinical condition changed to AECOPD. In addition, 120 healthy subjects were enrolled as healthy controls (HCs) at the same period. And the HCs were defined as the subjects who presented no obvious abnormalities in biochemical indexes, no history of respiratory diseases (such as COPD, asthma, tuberculosis, and bronchiectasis), hematological malignancies, solid tumors, autoimmune diseases, or severe infections. The study was approved by the Institutional Review Board of our hospital. All participants or their guardians signed the informed consents before enrollment.

2.2 | Data collection

The baseline data of all participants were recorded after enrollment, which included age, gender, body mass index (BMI), family history of chronic obstructive pulmonary disease (COPD), and history of smoke. The forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were measured after enrollment, and the ratio of these two measurements (FEV_1/FVC (%)) was calculated. FEV_1 (%predicted) was defined as the percentage of FEV_1 and predicted FEV_1. The airflow obstruction severity of COPD patients was defined according to GOLD guidelines as follows[15]: (1) GOLD 1 (mild): $FEV_1 \geq 80\%$ predicted; (2) GOLD 2 (moderate): $50\% \leq FEV_1 < 80\%$ predicted; (3) GOLD 3 (severe):

30% ≤ FEV_1 < 50% predicted; and (4) GOLD 4 (very severe): FEV_1 < 30% predicted.

2.3 | Sample collection

Peripheral blood sample of AECOPD patients and stable COPD patients were collected within 24 hours after enrollment, and peripheral blood sample of HCs was collected on the enrollment. After collection, the peripheral blood sample was centrifuged at 1000 *g* for 15 minutes (4°C), and then, the supernatant was subsequently isolated and further centrifuged at 10 000 g for 10 minutes (4°C). Finally, plasma was acquired and stored at −80°C until further detection.

2.4 | Enzyme-linked immunosorbent assay (ELISA)

The level of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, IL-17, and IL-23 in plasma was measured with the use of commercial human ELISA kits (Thermo Fisher Scientific). All procedures were carried out according to the kit instructions as follows: Firstly, plasma samples were added to the coated wells to bind to the immobilized antibody. After removal of unbonded antibody, a second antibody was added to complete the four-member sandwich. Finally, tetramethylbenzidine substrate solution was added to the wells. And the intensity was measured at 450 nm wavelengths on microplate reader (BioTek).

2.5 | Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The relative expressions of lncRNA MALAT1 and its target miRNAs (miR-125b, miR-133, miR-146a, and miR-203) in plasma were detected by RT-qPCR. Initially, total RNA was isolated from plasma using TRIzol™ Reagent (Thermo Fisher Scientific). Then, total RNA was reverse transcribed into complementary DNA using iScript™ cDNA Synthesis Kit (Bio-Rad). Subsequently, qPCR was conducted using KOD SYBR® qPCR Mix (Toyobo). The relative expressions of lncRNA and miRNA were calculated by $2^{-\triangle\triangle Ct}$ method using GAPDH as internal reference for lncRNA MALAT1 and U6 as internal reference for target miRNAs. The detailed description of $2^{-\triangle\triangle Ct}$ method calculation was as follow: ΔCt (test) = Ct (target, test) − Ct (reference, test), ΔCt (calibrator) = Ct (target, calibrator) − Ct (reference, calibrator), ΔΔCt = ΔCt (test) − ΔCt (calibrator), then the relative expressions of target genes (lncRNA MALAT1 and its target miRNAs) were calculated as $2^{-\Delta\Delta Ct}$. Sequences of primers used were shown in Table S1. Notably, it is reported that lncRNA MALAT1 sponges miR-125b, miR-133, miR-146a, or miR-203 to promote inflammation[2,4,12-14]; hence, miR-125b, miR-133, miR-146a, and miR-203 were selected as target miRNAs for lncRNA.

2.6 | Statistical analysis

Statistical analysis was performed using SPSS 22.0 (SPSS Inc), and figure was plotted using GraphPad Prism 7.00 (GraphPad Software Inc). Continuous variable was expressed as mean ± standard deviation (SD) or median and interquartile range (IQR), and categorical variable was described as count and percentage. Comparisons among three groups were determined by one-way analysis of variance (ANOVA), chi-square test, or Kruskal-Wallis H rank sum test. Comparison between two groups was determined by Wilcoxon rank sum test. Multiple comparisons were determined by Dunnett's test. Correlation between two variables was analyzed by Spearman's rank correlation test. Receiver operating characteristic (ROC) curve and area under the curve (AUC) with 95% confidence interval (CI) were used to discriminate AECOPD patients from stable COPD patients or HCs. According to ROC analysis, the best statistical cutoff value of lncRNA MALAT1 relative expression was calculated, which corresponds to the point (best cutoff point) at which the sum of false positives and false negatives was less than any other point. Then, the sensitivity and specificity of the best cutoff point was assessed. *P* value < .05 was considered significant.

3 | RESULTS

3.1 | Clinical characteristics

No difference of age (*P* = .809), gender (*P* = .754), or BMI (*P* = .302) was displayed among HCs, stable COPD, and AECOPD patients. The mean age was 67.0 ± 7.3 years in HCs, 66.5 ± 7.2 years in stable COPD patients, and 67.0 ± 6.9 years in AECOPD patients. And there were 37 (30.8%) females and 83 (69.2%) males in HCs, 36 (30.0%) females and 84 (70.0%) males in stable COPD patients, and 32 (26.7%) females and 88 (73.3%) males in AECOPD patients. In terms of family history of COPD, one-way ANOVA analysis followed by chi-square test showed that the rate of family history of COPD was the highest in AECOPD patients, followed by stable COPD patients, and then HCs (*P* = .030). Regarding history of smoke, one-way ANOVA analysis followed by chi-square test exhibited that the rate of history of smoke was the highest in stable COPD patients, then higher in AECOPD patients, and lowest in HCs (*P* < .001). As for lung function indexes, one-way ANOVA analysis followed by chi-square test displayed that the mean FEV_1/FVC (%) and mean FEV_1 (%predicted) were the lowest in AECOPD patients, followed by stable COPD patients, and then HCs (both *P* < .001). Furthermore, one-way ANOVA analysis followed by Kruskal-Wallis H rank sum test disclosed that the median levels of key inflammatory cytokines (including TNF-α, IL-1β, IL-6, IL-8, IL-17, and IL-23) were all the highest in AECOPD patients, higher in stable COPD patients, and the lowest in HCs (all *P* < .001). Additionally, disease severity was different between stable COPD and AECOPD patients (*P* = .044). The detailed information of characteristics was listed in Table 1.

TABLE 1 Comparison of clinical characteristics

Items	HCs (N = 120)	Stable COPD (N = 120)	AECOPD (N = 120)	*P* value
Demography characteristics				
Age (years), mean ± SD	67.0 ± 7.3	66.5 ± 7.2	67.0 ± 6.9	.809
Gender, No. (%)				.754
Female	37 (30.8)	36 (30.0)	32 (26.7)	
Male	83 (69.2)	84 (70.0)	88 (73.3)	
BMI (kg/m^2), mean ± SD	22.7 ± 2.6	22.2 ± 2.9	22.6 ± 3.0	.302
Family history of COPD, No. (%)				.030
No	100 (83.3)	87 (72.5)	83 (69.2)	
Yes	20 (16.7)	33 (27.5)	37 (30.8)	
History of smoke, No. (%)				<.001
No	86 (71.7)	58 (48.3)	62 (51.7)	
Yes	34 (28.3)	62 (51.7)	58 (48.3)	
Lung function indexes				
FEV_1/FVC (%), mean ± SD	82.1 ± 3.7	60.0 ± 6.3	58.7 ± 8.1	<.001
FEV_1 (%predicted), mean ± SD	98.9 ± 4.0	66.9 ± 17.7	60.2 ± 18.2	<.001
Disease severity				
GOLD stage, No. (%)				.044
1	-	49 (40.8)	36 (30.0)	
2	-	47 (39.2)	50 (41.7)	
3	-	24 (20.0)	31 (25.8)	
4	-	0 (0.0)	3 (2.5)	
Inflammatory cytokines				
TNF-α (pg/mL), median (IQR)	13.5 (8.5-20.9)	19.6 (10.6-32.6)	59.9 (34.0-85.2)	<.001
IL-1β (pg/mL), median (IQR)	0.9 (0.5-1.4)	1.4 (0.7-2.1)	3.9 (2.1-5.4)	<.001
IL-6 (pg/mL), median (IQR)	8.3 (4.0-11.7)	8.9 (5.0-18.8)	38.8 (15.5-55.5)	<.001
IL-8 (pg/mL), median (IQR)	12.3 (7.0-23.7)	20.9 (8.2-53.9)	54.5 (29.3-129.9)	<.001
IL-17 (pg/mL), median (IQR)	10.1 (6.1-17.1)	14.7 (6.7-35.1)	50.5 (23.9-103.6)	<.001
IL-23 (pg/mL), median (IQR)	37.7 (19.7-68.0)	71.8 (24.0-145.7)	184.9 (95.1-396.0)	<.001

Note: Comparison was determined by one-way analysis of variance (ANOVA), chi-square test, Kruskal-Wallis H rank sum test, or Wilcoxon rank sum test.

Abbreviations: AECOPD, acute exacerbation chronic obstructive pulmonary disease; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HCs, healthy controls; IL, interleukin; IQR, interquartile range; SD, standard deviation; TNF-α, tumor necrosis factor-α.

3.2 | The predictive value of lncRNA MALAT1 for AECOPD risk

One-way ANOVA analysis followed by Dunnett's test exhibited that lncRNA MALAT1 was higher in AECOPD patients (3.299 (2.250-4.436)) than that in stable COPD patients (1.883 (1.390-2.335)) and HCs (1.019 (0.450-1.560)) (both $P < .001$) (Figure 1A). Further ROC curve analysis disclosed that lncRNA MALAT1 was of an excellent value for distinguishing AECOPD patients from HCs (AUC: 0.969, 95% CI: 0.951-0.987), and the sensitivity and the specificity were 99.2% and 83.3%,

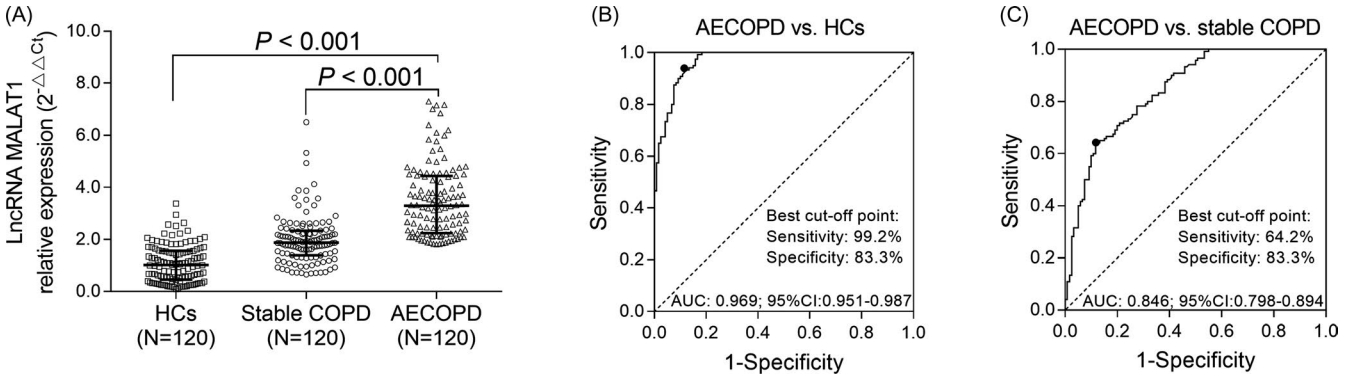

FIGURE 1 The value of lncRNA MALAT1 for predicting AECOPD risk. Comparison of lncRNA MALAT1 relative expression between AECOPD patients vs HCs, AECOPD patients vs stable COPD patients (A). The ROC curves of lncRNA MALAT1 in distinguishing AECOPD patients from HCs (B) and from stable COPD patients (C). LncRNA MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; AECOPD, acute exacerbation chronic obstructive pulmonary disease; HCs, healthy controls; COPD, chronic obstructive pulmonary disease; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval

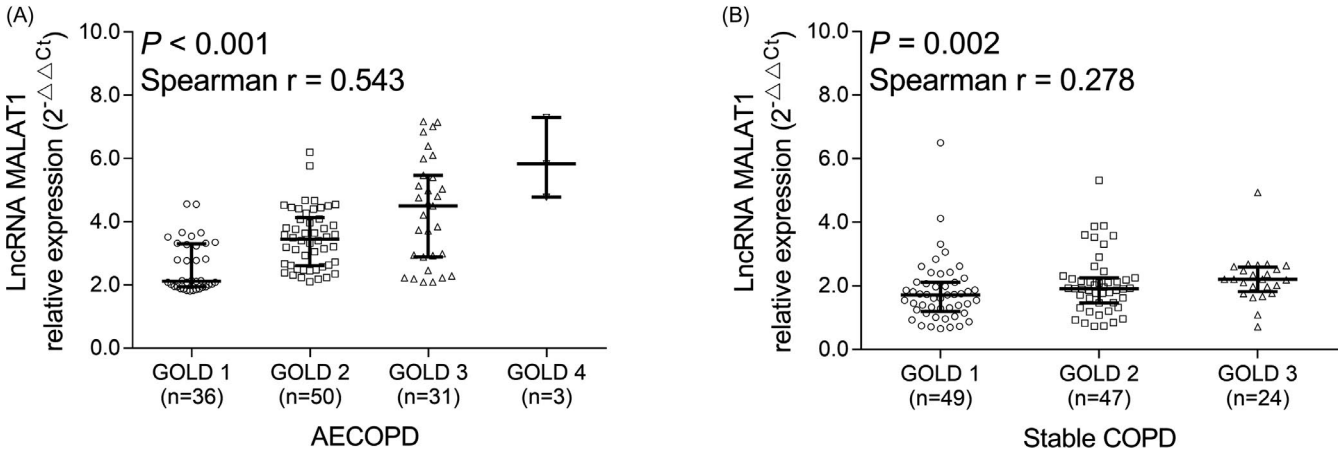

FIGURE 2 LncRNA MALAT1 in stable COPD and AECOPD patients with different GOLD stage. Correlation of lncRNA MALAT1 with GOLD stage in stable COPD patients (A) and in AECOPD patients (B). LncRNA MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; COPD, chronic obstructive pulmonary disease; AECOPD, acute exacerbation chronic obstructive pulmonary disease; GOLD, global initiative for chronic obstructive lung disease

respectively, at the best cutoff point (the large sum of sensitivity and specificity) (Figure 1B). Furthermore, lncRNA MALAT1 presented a good value for differentiating AECOPD patients from stable COPD patients (AUC: 0.846, 95% CI: 0.798-0.894), and the sensitivity and the specificity were 64.2% and 83.3%, respectively, at the best cutoff point (the large sum of sensitivity and specificity) (Figure 1C). These findings implied that lncRNA MALAT1 exhibited a good predictive value for AECOPD risk.

3.3 | Correlation of lncRNA MALAT1 with disease severity

In AECOPD patients, lncRNA MALAT1 relative expression was the highest in GOLD stage 4 patients (5.837 (4.779-incalculable)), followed by GOLD stage 3 patients ((4.506 (2.890-5.467)) and GOLD stage 2 patients ((3.452 (2.609-4.132)), and then GOLD stage 1 patients (2.124 (1.946-3.308)) ($P < .001$) (Figure 2A). As for stable COPD patients, lncRNA MALAT1 relative expression was highest in GOLD stage 3 patients ((2.200 (1.820-2.587)), followed by GOLD stage 2 patients ((1.913 (1.459-2.257)), and then GOLD stage 1 patients ((1.714 (1.194-2.115)) ($P = .002$) (Figure 2B).

3.4 | Correlation of lncRNA MALAT1 with inflammation

In AECOPD patients, lncRNA MALAT1 positively correlated with TNF-α ($P < .001$, $r = .445$), IL-1β ($P < .001$, $r = .359$), IL-6 ($P < .001$, $r = .360$), IL-8 ($P < .001$, $r = .368$), IL-17 ($P < .001$, $r = .497$), and IL-23 ($P < .001$, $r = .444$) (Table 2). In stable COPD patients, lncRNA MALAT1 was positively associated with TNF-α ($P < .001$, $r = .367$), IL-1β ($P < .001$, $r = .346$), IL-6 ($P = .002$, $r = .274$), IL-8 ($P < .001$, $r = .349$), IL-17 ($P < .001$, $r = .429$), and IL-23 ($P < .001$, $r = .434$)

TABLE 2 Correlation of lncRNA MALAT1 with inflammatory cytokines

	LncRNA MALAT1 in HCs		LncRNA MALAT1 in stable COPD		LncRNA MALAT1 in AECOPD	
Items	Spearman *r*	*P* value	Spearman *r*	*P* value	Spearman *r*	*P* value
TNF-α	.225	.014	.367	<.001	.445	<.001
IL-1β	.116	.207	.346	<.001	.359	<.001
IL-6	.200	.029	.274	.002	.360	<.001
IL-8	.205	.024	.349	<.001	.368	<.001
IL-17	.193	.035	.429	<.001	.497	<.001
IL-23	.139	.130	.434	<.001	.444	<.001

Note: Correlation was analyzed by Spearman's rank correlation test.

Abbreviations: AECOPD, acute exacerbation chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease; HCs, healthy controls; IL, interleukin; IQR, interquartile range; TNF-α, tumor necrosis factor-α.

TABLE 3 Correlation of lncRNA MALAT1 with candidate miRNAs

	LncRNA MALAT1 in HCs		LncRNA MALAT1 in stable COPD		LncRNA MALAT1 in AECOPD	
Items	Spearman *r*	*P* value	Spearman *r*	*P* value	Spearman *r*	*P* value
MiR-125b	−.247	.7	−.264	.4	−.333	<.1
MiR-133	−.242	.8	−.111	.229	−.162	.76
MiR-146a	−.22	.27	−.251	.6	−.35	.1
MiR-203	−.145	.115	−.128	.164	−.27	.3

Note: Correlation was analyzed by Spearman's rank correlation test.

Abbreviations: AECOPD, acute exacerbation chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease; HCs, healthy controls; miRNAs, microRNAs.

(Table 2). As for HCs, lncRNA MALAT1 positively correlated with TNF-α (P = .014, r = .225), IL-6 (P = .029, r = .200), IL-8 (P = .024, r = .205), and IL-17 (P = .035, r = .193), whereas no correlation of lncRNA MALAT1 with IL-1β (P = .207, r = .116) and IL-23 (P = .130, r = .139) was observed (Table 2). Notably, the correlation coefficients of lncRNA MALAT1 with inflammatory cytokines were numerically highest in AECOPD patients, while relatively lower in stable COPD patients and HCs.

3.5 | Correlation of lncRNA MALAT1 with miR-125b, miR-133, miR-146a, and miR-203

In AECOPD patients, lncRNA MALAT1 was negatively correlated with miR-125b (P < .001, r = −.333), miR-146a (P = .001, r = −.305), and miR-203 (P = .003, r = −.270), but not with miR-133 (P = .076, r = −.162) (Table 3). In stable COPD patients, lncRNA MALAT1 was negatively correlated with miR-125b (P = .004, r = −.264) and miR-146a (P = .006, r = −.251), but not with miR-133 (P = .229, r = −.111) and miR-203 (P = .164, r = −.128) (Table 3). Regarding HCs, lncRNA MALAT1 was negatively associated with miR-125b (P = .007, r = −.247), miR-133 (P = .008, r = −.242), and miR-146a (P = .027, r = −.202), but not with miR-203 (P = .115, r = −.145) (Table 3).

3.6 | Clinical implication of candidate target miRNAs in COPD

One-way ANOVA analysis followed by Kruskal-Wallis H rank sum test displayed that miR-125b, miR-133, miR-146a, and miR-203 were the lowest in AECOPD patients, followed by stable COPD patients, and then HCs (all P < .001) (Table 4). Besides, miR-125b, miR-133, miR-146a, and miR-203 negatively correlated with inflammation and disease severity in AECOPD and stable COPD patients (mostly P < .05), with the detailed information exhibited in Table 5.

4 | DISCUSSION

In order to explore the implication of lncRNA MALAT1 in the development of AECOPD, we compared the lncRNA MALAT1 relative expression among HCs, stable COPD, and AECOPD patients as well as performed a further ROC curve analysis of lncRNA MALAT1 for distinguishing AECOPD patients from HCs and stable COPD patients. The findings provided the first clinical evidence that lncRNA MALAT1 was increased in AECOPD patients compared with HCs and stable COPD, and it had good values for distinguishing AECOPD patients from stable COPD patients

TABLE 4 Comparison of candidate miRNAs

MiRNAs	HCs (N = 120)	Stable COPD (N = 120)	AECOPD (N = 120)	*P* value
MiR-125b	1.047 (0.615-2.287)	0.872 (0.595-1.561)	0.443 (0.290-0.745)	<.001
MiR-133	1.472 (0.724-3.162)	1.228 (0.679-1.902)	0.620 (0.357-1.135)	<.001
MiR-146a	1.346 (0.716-2.490)	1.033 (0.650-1.705)	0.559 (0.273-0.974)	<.001
MiR-203	1.512 (0.865-3.134)	1.263 (0.729-2.170)	0.672 (0.361-1.272)	<.001

Note: Comparison was determined by Kruskal-Wallis H rank sum test.

Abbreviations: AECOPD, acute exacerbation chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease; HCs, healthy controls; miRNAs, microRNAs.

TABLE 5 Correlation of candidate miRNAs with GOLD stage and inflammatory cytokines

		HCs				Stable COPD				AECOPD			
Items		MiR-125b	MiR-133	MiR-146a	MiR-203	MiR-125b	MiR-133	MiR-146a	MiR-203	MiR-125b	MiR-133	MiR-146a	MiR-203
GOLD stage	Spearman *r*	-	-	-	-	−.154	−.162	−.238	−.172	−.476	−.298	−.501	−.477
	P value	-	-	-	-	.092	.077	.009	.061	<.001	.001	<.001	<.001
TNF-α	Spearman *r*	−.127	−.136	−.180	−.184	−.258	−.205	−.313	−.074	−.247	−.104	−.258	−.229
	P value	.166	.139	.050	.045	.004	.025	.001	.420	.006	.259	.004	.012
IL-1β	Spearman *r*	−.136	−.134	−.169	−.146	−.194	−.142	−.199	−.016	−.295	−.143	−.198	−.212
	P value	.138	.145	.065	.111	.033	.122	.029	.860	.001	.120	.030	.020
IL-6	Spearman *r*	−.261	−.183	−.312	−.298	−.192	−.174	−.349	−.138	−.167	−.024	−.157	−.142
	P value	.004	.046	.001	.001	.035	.057	<.001	.133	.069	.796	.088	.121
IL-8	Spearman *r*	−.122	−.149	−.177	−.147	−.258	−.115	−.312	−.207	−.152	−.007	−.083	−.203
	P value	.184	.104	.053	.109	.004	.212	.001	.023	.098	.936	.368	.026
IL-17	Spearman *r*	−.196	−.213	−.191	−.230	−.301	−.191	−.342	−.279	−.196	−.124	−.211	−.282
	P value	.032	.019	.037	.012	.001	.037	<.001	.002	.032	.176	.021	.002
IL-23	Spearman *r*	−.216	−.195	−.189	−.238	−.284	−.196	−.343	−.251	−.170	−.116	−.193	−.287
	P value	.018	.033	.039	.009	.002	.032	<.001	.006	.063	.207	.035	.002

Note: Correlation was analyzed by Spearman's rank correlation test.

Abbreviations: AECOPD, acute exacerbation chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HCs, healthy controls; IL, interleukin.; IQR, interquartile range; miRNAs, microRNAs; *r*, Correlation coefficient; TNF-α, tumor necrosis factor-α.

(AUC: 0.846, 95% CI: 0.798-0.894) and from HCs (AUC: 0.969, 95% CI: 0.951-0.987). Herein, we proposed several explanations: (1) LncRNA MALAT1 might facilitate the release of inflammatory cytokines (such as TNF-α and IL-6) and amplify the inflammation, thus resulted in elevated AECOPD risk. (2) LncRNA MALAT1 might cause tissue damage and lung injury by regulating its downstream pathways (such as p38 MAPK/p65 NF-κB signaling pathway), which contributed to the exacerbation of COPD. Our finding was supported by the results of other mechanism studies that lncRNA MALAT1 stimulates inflammation and amplifies lung injury, which are vital in the progression and exacerbation of COPD.[11,16,17] For instance, lncRNA MALAT1 induces the release of inflammatory mediators TNF-α and IL-6 through activating serum amyloid antigen 3 in the endothelial cells.[16] Another study displays that knockdown of lncRNA MALAT1 represses the production of inflammatory cytokines via inhibiting the p38 MAPK/p65 NF-κB signaling pathway in lung tissues, which improves the LPS-induced lung injury.[17] In addition, a recent clinical study illustrates that lncRNA MALAT1 expression is higher in sepsis patients than that in HCs and it exhibited a good predictive value for sepsis risk.[14]

The present study also exhibited that lncRNA MALAT1 was positively associated with GOLD stage and inflammation cytokines (including TNF-α, IL-1β, IL-6, IL-8, IL-17, and IL-23) in both stable COPD and AECOPD patients. These could be explained by (1) lncRNA MALAT1 might induce inappropriate immune response and excessive inflammatory reasons via mediating related signaling pathway such as p38 MAPK/p65 NF-κB, which was responsible for prolonged inflammation and increased disease severity in stable COPD and AECOPD patients, and (2) lncRNA MALAT1 might amplify pro-inflammatory gene expression and protein release as well as oxidative tissue injury, thus led to lung injury and accelerated disease severity in stable COPD and AECOPD

patients. Notably, the correlation coefficient values of lncRNA MALAT1 with inflammatory cytokines in HCs were all below 0.3, which indicated that the correlation of lncRNA MALAT1 with inflammation was weak in HCs. As for COPD patients, the correlation of lncRNA MALAT1 with inflammatory cytokines was strongest in AECOPD patients, followed by stable COPD patients. The possible reason was as follows: During exacerbations, inflammation cascade was evoked to further induce epigenetic dysregulation and intensify gene transcription of the inflammatory cytokines associated with airway inflammation via activating transcription factors, which further promoted the pro-inflammatory environment in alveolar macrophages.[8,18] Thereby, the regulation of inflammatory cytokines by lncRNA MALAT1 was more prominent in AECOPD patients.

As lncRNA MALAT1 is recently introduced as a competing endogenous RNA of miR-125b, miR-133, miR-146a, and miR-203 to stimulate inflammatory responses, therefore, we conduced further analysis to investigate the correlation of lncRNA MALAT1 with its target microRNAs (miR-125b, miR-133, miR-146a, and 13miR-203) in COPD. The findings were that lncRNA MALAT1 negatively correlated with miR-125b, miR-146a, and miR-203 in AECOPD patients, miR-125b and miR-146a in stable COPD patients, and miR-125b, miR-133, and miR-146a in HCs. Furthermore, miR-125b, miR-133, miR-146a, and miR-203 were the lowest in AECOPD patients, followed by stable COPD patients, and then in HCs, and they were negatively associated with inflammation and disease severity in stable COPD and AECOPD patients. In accordance with prior studies, one study based on an acute lung injury rat displays that lncRNA MALAT1 overexpression attenuates LPS-induced inflammatory response in murine alveolar macrophages by sponging miR-146a.[2] Another study based on rat sepsis model exhibits that lncRNA MALAT1 enhances cardiac inflammation dysfunction via interacting with miR-125b and p38 MAPK/NFκB in sepsis.[4] These findings suggested that lncRNA MALAT1 might interact with miR-125b, miR-146a, or/and miR-203 to promote the inflammation and accelerate the disease severity in COPD. However, the detailed mechanisms of miR-125b, miR-146a, or/and miR-203 underlying COPD was not explored; further, experiment studies would be desirable for validate our speculation.

Some limitations needed to be taken into consideration in our study. Firstly, the sample size was relatively small, which might reduce the statistic power. Thereby, large sample size needed for further validation of the findings. Secondly, the molecular mechanism of lncRNA MALAT1 in the development and progression of AECOPD was not investigated. Thirdly, blood samples of AECOPD patients were obtained within 24 hours after enrollment; however, time intervals from the onset of AECOPD to hospital admission were variable among patients, which might cause bias to our results. Lastly, the effect of lncRNA MALAT1 on short-term and long-term prognosis was not explored in stable COPD and AECOPD patients.

In conclusion, lncRNA MALAT1 holds the potential as a biomarker for predicting the acute exacerbation risk and disease progression of COPD via the inner-correlation with its targets miR-125b, miR-146a, and miR-203, which might facilitate a more precise and effective disease management.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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