Original Article

Exploring the Clinical Significance and Mechanistic Role of the LINC00487/hsa-miR-663b Axis in Cell Line Models of Acute Lung Injury

(long non-coding RNA / microRNA / acute lung injury / biomarkers)

XIXIANG YAN[1], ZIQI XIE[2], XIAOYE ZHENG[2], QING XIE[2], JUNLE YANG[3], XIAOJUAN WU[2]

[1]Respiratory and Critical Care Medicine, Affiliated Jianhu Hospital of Xinglin College, Nantong University, Nantong, China
[2]Department of Ultrasound Medicine, The Affiliated Hospital of Northwest University (Xi'an No.3 Hospital), Xi'an, China
[3]Department of Imaging Medicine, The Affiliated Hospital of Northwest University (Xi'an No.3 Hospital), Xi'an, China

Abstract. Acute lung injury (ALI) is a serious lung disease that tends to progress to acute respiratory distress syndrome (ARDS). This study was aimed to seek new biomarkers of ALI to provide a basis for monitoring the progress of ALI in time. A human bronchial epithelial cell line (HBEC3-KT) was treated with 1 μg/ml lipopolysaccharide (LPS) to induce the ALI response. The expression of LINC00487 and hsa-miR-663b in LPS-treated HBEC3-KT cells was detected by RT-qPCR. The regulation of hsa-miR-663b by LINC00487 was investigated using a dual luciferase assay and an over-expression experiment. Cell proliferation and apoptosis were detected by the CCK-8 assay and annexin V-FITC kit. Serum levels of LINC00487 and hsa-miR-663b were detected by collecting blood samples from ALI patients (with or without ARDS), and the ROC curve was constructed to assess their clinical value in ALI. LPS inhibited proliferation of HBEC3-KT cells and promoted their apoptosis and inflammatory response, which were further enhanced by LINC00487 over-expression and reversed by an hsa-miR-663b mimic. The hsa-miR-663b mimic weakened the luciferase activity of HBEC3-KT cells transfected with the luciferase vector of wild-type LINC00487. The cellular level of hsa-miR-663b was down-regulated by LINC00487 over-expression and increased by LINC00487 knockdown. The ROC curve showed that LINC00487 combined with hsa-miR-663b effectively diagnosed ALI (AUC = 0.840) and was a classifier for ALI patients with or without ARDS (AUC = 0.822). Serum LINC00487 and hsa-miR-663b levels are valuable biomarkers of ALI and can monitor the ALI progress. LINC00487 may promote ALI progression by negatively regulating hsa-miR-663b.

Introduction

Acute lung injury (ALI) is a common acute respiratory disease in clinical practice, with pathological features of dysregulation of ventilation/blood flow ratio, reduced lung compliance, reduced lung volume, and other consequences (Long et al., 2022). ALI is one of the fatal complications of respiratory virus infections such as coronavirus, accompanied by acute severe inflammatory reactions in the lung (Zhu et al., 2023). Severe ALI is prone to acute respiratory distress syndrome (ARDS), manifesting as hypoxaemia, acute onset, respiratory distress and high fatality rate. In the United States, the mortality rates for ALI and ARDS patients are 38.5 vs and 41.1 %, respectively (Suresh et al., 2000). A global observational study of 145 paediatric intensive care units (ICUs) across 27 countries reported morbidity and mortality rates of 17 % and 3.2 % in 23,280 ARDS patients (Khemani et al., 2019).

ALI is an injury of alveolar epithelial cells and capillary endothelial cells caused by various injury factors,

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Corresponding author: Xiaojuan Wu, Department of Ultrasound Medicine, The Affiliated Hospital of Northwest University (Xi'an No.3 Hospital), No.10 East Section of Fengcheng 3rd Road, Weiyang District, Xi'an 710008, Shaanxi Province, China. Phone: (+86) 029-618 16204; E-mail: xiaojuanwu8632@163.com

Abbreviations: ALI – acute lung injury, ARDS – acute respiratory distress syndrome, AUC – area under the curve, GEO – Gene Expression Omnibus, ICUs – intensive care units, lncRNA – long non-coding RNA, LPS – lipopolysaccharide, miRNA – microRNA, OD – optical density, PBS – phosphate-buffered saline.

resulting in diffuse pulmonary interstitial and alveolar oedema, thereby leading to acute hypoxic respiratory insufficiency (Hu et al., 2022). At present, the diagnostic criteria for ALI are the presence of pathogenic factors; oxygenation index (arterial partial pressure of oxygen/inhaled oxygen concentration, PaO2/Fi02) < 300 mmHg; bilateral chest infiltration on X-ray; acute episodic respiratory failure (Yang et al., 2023). However, with the deepening of ALI research, this diagnostic standard still has some shortcomings.

The studies of biomarkers have now penetrated a variety of human diseases. Mining effective biomarkers will enable a more accurate diagnosis of ALI and monitoring its progress so that relevant medical measures can be taken in time. The roles of long non-coding RNA (lncRNA) and microRNA (miRNA) in ALI, both of which are potential biomarkers of ALI, have been extensively studied. ALI is a common and serious complication of sepsis. The expression level of lncRNA H19 was low in the serum samples of sepsis patients, and the area under the curve (AUC) of H19 to diagnose sepsis patients with or without ALI was 0.9141 (95 % CI, 0.87–0.96) (Zhou et al., 2022). Wei and Liu (2019) showed that lncRNA MALAT1 can aggravate sepsis-induced inflammation by modulating the p38 MAPK/p65 NF-κB signalling pathway. miR-29a-3p was shown to be down-regulated in the plasma of ARDS patients and the lung tissues of ALI mice. Injection of miR-29a-3p can improve lung injury in ALI mice by reducing PANoptosis of alveolar epithelial cells and inhibiting expression of inflammatory factors in the lung (Cui et al., 2022). Liu et al. (2021b) showed that miR-384-5p is enriched in the bone marrow mesenchymal stem cell-derived exosomes, which attenuates LPS-induced apoptosis and viability loss in alveolar macrophages and improves 7-day survival in ALI rats.

Based on the important functions of lncRNAs and miRNAs in the progression of ALI, we screened out another potential lncRNA (LINC00487) related to ALI by analysing the public datasets (GSE157103 and GSE243066), and we predicted hsa-miR-663b as the downstream target of LINC00487 through the lncRNASNP2 online database. As a novel lncRNA in ALI, the function of LINC00487 has not been reported. The competitive endogenous RNA (ceRNA) network of ALI constructed by Liu et al. (2021a) revealed the central position of miR-663b, but its specific role and value have not yet been studied. Here, we explored the potential functions of LINC00487 and hsa-miR-663b in the progression of ALI and evaluated their diagnostic value for ALI.

Material and Methods

Databases

The Gene Expression Omnibus (GEO) database is the source of the GSE157103 and GSE243066 datasets and is available at: https://www.ncbi.nlm.nih.gov/geo/. GSE157103 is a gene expression profile obtained through high-throughput sequencing analysis based on whole blood leukocyte samples from COVID-19 patients and non-COVID-19 individuals. GSE243066 is an mRNA and miRNA sequencing analysis based on whole blood samples from ARDS patients and healthy donors. The Human microRNA Disease Database (HMDD v4.0) (http://www.cuilab.cn/hmdd/) was used to collect ALI-related miRNAs in this study. Downstream miRNAs of LINC00487 were predicted in the lncRNASNP2 database (https://guolab.wchscu.cn/lncRNASNP#!/).

Cell line and treatment

The immortalized HBEC3-KT cell line purchased from the American Type Culture Collection was maintained in a human bronchial epithelial complete culture medium (Pricella, Houston, TX). The cell line was cultured in a humid 37 °C environment with 5 % CO_2.

HBEC3-KT cells were treated with 1 μg/ml of lipopolysaccharide (LPS) (Sigma, Burlington, MA) for 24 hours to induce the ALI response. Phosphate-buffered saline (PBS) was used as a control.

Real time-quantitative PCR (RT-qPCR)

Total RNA was extracted from cells using TRIzol (Beyotime,Shanghai, China). PCR was performed in an ABI 7500 system using a ThermoFisher (Waltham, MA) DyNAmo HS SYBR Green qPCR kit. Finally, the $2^{-\Delta\Delta CT}$ method was used to calculate the fold change of RNA relative expression. GAPDH was used for the internal control of LINC00487 and mRNA, and U6 was the internal reference of hsa-miR-663b.

Cell transfection

One day before transfection, HBEC3-KT cells were inoculated into a 6- or 96-well plate. The next day, DNA or RNA transfection was carried out when the cell confluence reached about 70–80 % or 30–40 %. Thirty minutes before transfection, the complete medium was replaced with 1.75 ml/190 μl (6-/96-well plate) of serum-free medium. The amount of 125/5 μl of Opti-MEM medium (Yobibio, Shanghai, China) was added to each of two sterile centrifuge tubes. The amount of 2.5/0.2 μg of plasmid or 100/5 pmol of RNA was added to one tube and 6/0.4 μl of Lipofectamine 3000 (ThermoFisher) was added to the other tube. After gently mixing each, they were left to stand for 5 minutes. The two tubes of liquid were then mixed and left to stand at room temperature for 20 minutes. Then, the transfection mixture was added to the cells. A follow-up experiment was performed 6–8 hours later.

Cell viability assay

After transfection of cells according to the above method, the cells were treated with or without LPS for 24 hours. After the processing was completed, the culture medium was replaced with a normal growth culture medium. Cell proliferation was detected using the CCK-8 reagent (Solarbio, Beijing, China) at different time points. In short, a medium containing 10 % CCK-8 reagent was

added to each well. After incubation at 37 °C for 2 hours, the optical density (OD) value of each well at 450 nm wavelength was checked using an enzyme-labelling instrument.

Apoptosis detection

The Annexin V-FITC Apoptosis Staining kit (Abcam, Cambridge, UK) was used to stain the treated cells according to the instructions. In brief, the processed cells were digested with trypsin digestion solution and then collected in centrifuge tubes. After counting, 8×10^4 resuspended cells were taken and centrifuged (1000 *g*, 5 minutes), and the cell precipitates were collected. First, 195 µl of the binding solution was added to resuspend the cell precipitate gently. Then, 5 µl of annexin V-FITC and 10 µl of propidium iodide solution were added in sequence to mix gently. The cells were incubated at room temperature in the dark for 20 minutes. Finally, apoptotic cells were detected by CytoFLEX flow cytometry (Breckman Coulter, Brea, CA).

Dual luciferase assay

The hsa-miR-663b mimic and the luciferase vectors of wild-type/mutant LINC00487 were co-transfected into HBEC3-KT cells according to the above method. After 48 hours, the luciferase activity was detected according to the instructions of the Renilla-Firefly Luciferase Dual Assay kit (MedChemExpress, Monmouth Junction, NJ).

Volunteers and samples

In this study, we collected the blood samples of 40 healthy volunteers, 36 ALI patients who did not develop ARDS and 36 ARDS patients (Supplementary Table 1) from The Affiliated Hospital of Northwest University (Xi'an No.3 Hospital). The blood samples were left to stand at room temperature for 15 minutes and then centrifuged (1000 *g* for 10 minutes). After centrifugation, the supernatant in the collection tube represented the serum sample. The collected serum samples were subjected to RT-qPCR according to the above-mentioned method. All the volunteers have signed informed consent forms. This study has been approved by the Ethics Committee of The Affiliated Hospital of Northwest University (Xi'an No.3 Hospital, ethics approval number: 2020024).

Assessment of the clinical value of LINC00487 and hsa-miR-663b

The reliability of LINC00487 and hsa-miR-663b in diagnosing ALI and ARDS was evaluated through the ROC analysis using the GraphPad Prism 8.0 software. Multivariate binary logistic regression analysis was used to evaluate the influence of each factor on ALI progression with the aid of the SPSS software.

Statistical analysis

All the data in this study were replicated more than four times. The GraphPad Prism 8.0 software was applied to the disparity analysis of experimental data using the Student's *t*-test or one-way ANOVA method. A P value below 0.05 was statistically significant.

Results

Potential role of LINC00487 in the progression of ALI

Differential expression analysis of gene expression data in the GSE157103 and GSE243066 datasets was performed using the GEO2R function in the GEO database. By setting the screening criteria "$P < 0.05$ and $|\log2\ FC| > 2$", six and 55 lncRNAs were confirmed to be differentially expressed in the GSE157103 and GSE243066 datasets (Fig. 1A and Supplementary Table 2). Fig. 1B and C show the RNA expression profiles of the GSE157103 and GSE243066 datasets, respectively. Because LINC00487 showed a higher fold change in both datasets, with log2 FC of 3.29 and 3.55, we chose it as the object of this study.

HBEC3-KT cells were treated with 1 µg/ml LPS for 24 hours to simulate the injury response. LINC00487 was significantly up-regulated in LPS-treated HBEC3-KT cells (Fig. 1D). The over-expressed plasmid (op-LINC00487) and small interfering RNA (si-LINC00487) of LINC00487 were introduced to explore further mechanisms (Fig. 1E and F). The CCK-8 assay showed that LPS significantly inhibited the cell viability of HBEC3-KT. Over-expression of LINC00487 further reduced this viability, and LINC00487 knockdown rescued the LPS-induced decrease in cell viability (Fig. 1G). Pro-inflammatory factors IL-1 and TNF-α were significantly increased in the presence of LPS, while the anti-inflammatory factors IL-4 and TGF-β were decreased. The effects of LPS on inflammatory cytokines were enhanced by increased expression of LINC00487 and reversed by its inhibition (Fig. 1H and I). Moreover, LPS increased the apoptosis rate of HBEC3-KT cells, which was also enhanced by LINC00487 over-expression and reversed by its knockdown (Fig. 1J).

All of the above results suggest that LINC00487 is a potential risk factor for ALI, which impaired HBEC3-KT cell proliferation and promoted their inflammatory response and apoptosis.

Hsa-miR-663b is a downstream target of LINC00487

For further exploration, we searched the miRNAs closely related to ALT in the HMDD v4.0 database. Among them, hsa-miR-663b is the downstream miRNA of LINC00487 predicted in the lncRNASNP2 database (Fig. 2A and Supplementary Table 2). Figure 2B shows the binding sequence of LINC00487 and hsa-miR-663b. Based on this, we verified the interaction between them by constructing luciferase vectors. The results showed that transfection of hsa-miR-663b mimic (mim-663b) significantly up-regulated the expression of hsa-miR-663b

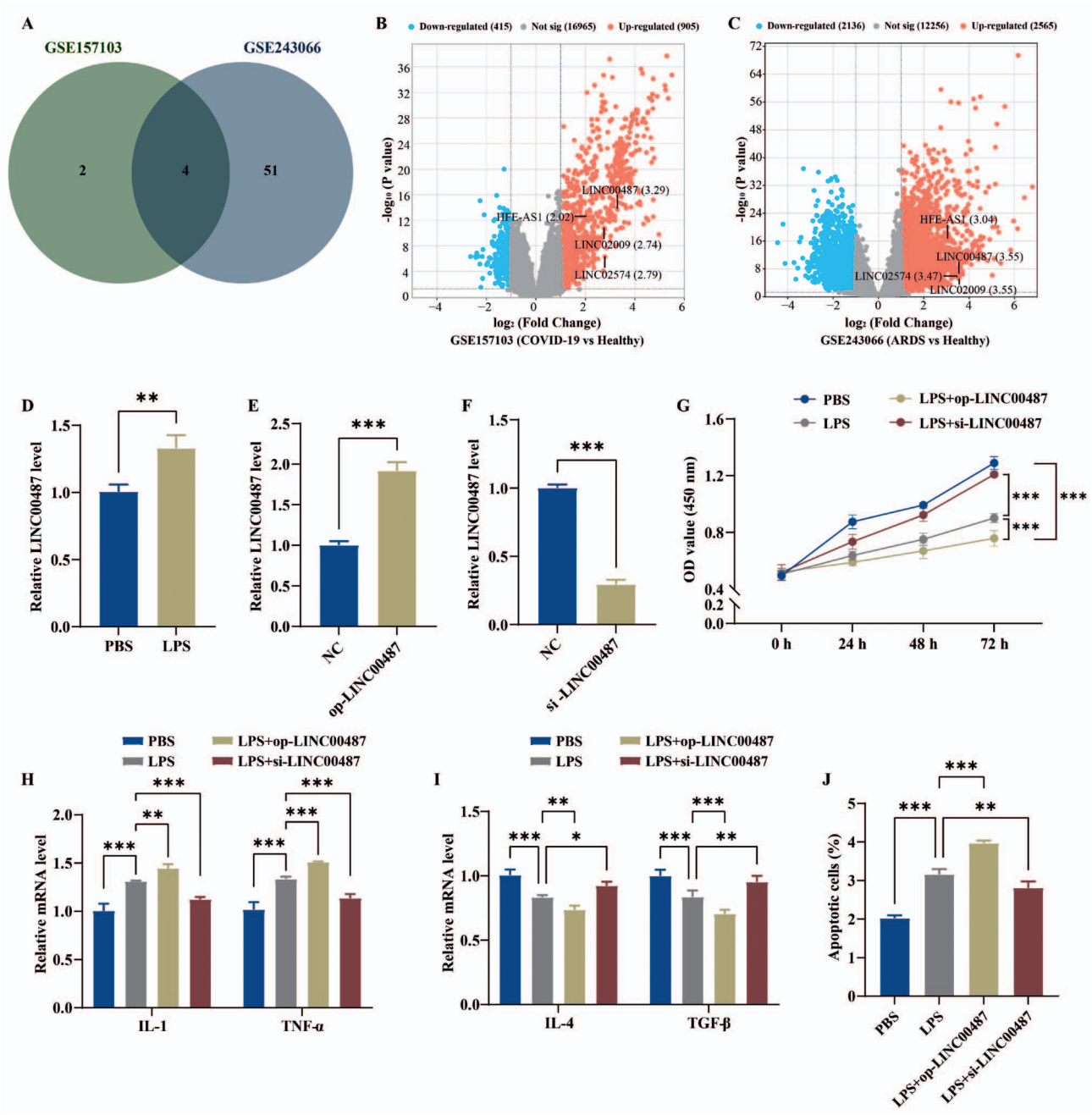


Fig. 1. LINC00487 in ALI. **(A)** Intersection of differentially expressed miRNAs in the GSE157103 and GSE243066 datasets. (**B** and **C**) miRNA expression profiles in GSE157103 and GSE243066 datasets. **(D)** Expression of LINC00487 in HBEC3-KT cells with LPS treatment. **(E** and **F)** Effect of op-LINC00487 or si-LINC00487 on the expression of LINC00487. Effects of LPS and LINC00487 on the proliferation **(G)**, inflammation **(H** and **I)** and apoptosis **(J)** of HBEC3-KT cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

(Fig. 2C), which inhibited the luciferase activity in cells transfected with the luciferase vector of wild-type LINC00487 (Fig. 2D). After over-expressing LINC00487 in HBEC3-KT cells, the cellular level of hsa-miR-663b was down-regulated (Fig. 2E) and the hsa-miR-663b level was increased by LINC00487 knockdown (Fig. 2E). These results suggest that LINC00487 negatively regulated the cellular level of hsa-miR-663b by binding to it.

Over-expression of hsa-miR-663b inhibits the function of LINC00487

The expression of hsa-miR-663b was deficient in LPS-treated HBEC3-KT cells (Fig. 3A). Transfection of the hsa-miR-663b mimic saved the decrease of cell viability induced by LPS, and LINC00487 over-expression counteracted this effect of hsa-miR-663b (Fig. 3B). Over-expression of LINC00487 intensified the LPS-

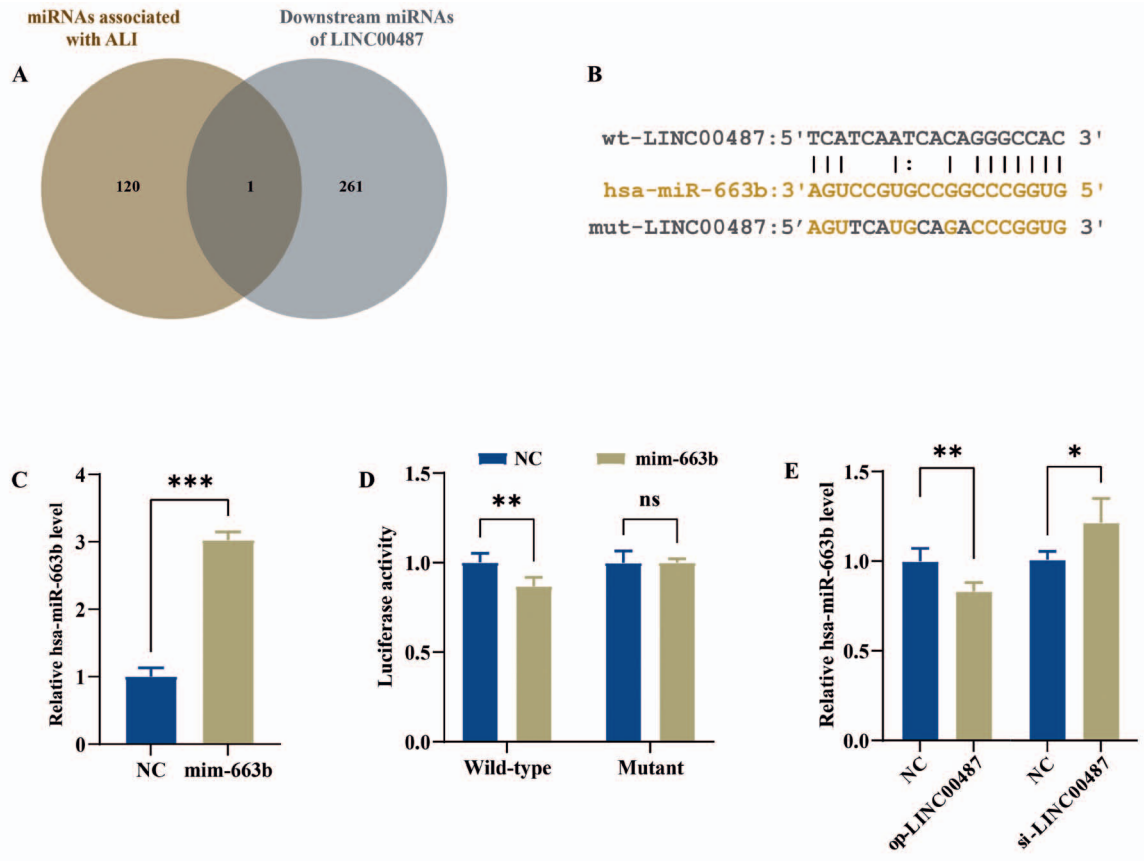


Fig. 2. Regulation of hsa-miR-663b by LINC00487. **(A)** Intersection of ALI-associated miRNAs and downstream miRNAs of LINC00487. **(B)** Binding sequence of LINC00487 targeting hsa-miR-663b. **(C)** Over-expression efficiency of hsa-miR-663b mimic. **(D)** Dual luciferase assay to verify the interaction between LINC00487 and hsa-miR-663b. **(E)** Effect of LINC00487 on the cellular level of hsa-miR-663b. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, no significant difference.

induced increase in HBEC3-KT apoptosis, which was reversed by the hsa-miR-663b mimic (Fig. 3C). Similarly, the hsa-miR-663b mimic also rescued the up-regulation of pro-inflammatory factors and down-regulation of anti-inflammatory factors caused by LPS and LINC00487 over-expression (Fig. 3D and E). These data suggest that hsa-miR-663b protected HBEC3-KT cells from the damage caused by LINC00487 over-expression and LPS.

Clinical value of the LINC00487/hsa-miR-663b axis in ALI

To investigate the clinical value of LINC00487 and hsa-miR-663b, the peripheral blood samples from 40 healthy volunteers, 36 patients with ALI (who did not develop ARDS) and 36 patients developing ARDS were collected for RT-qPCR. The serum LINC00487 levels in ALI patients were significantly higher than in healthy controls. With the progression of ALI, the serum LINC00487 level was further increased (ARDS patients vs ALI patients) (Fig. 4A). However, hsa-miR-663b was deficient in the serum of ALI patients and further decreased with the aggravation of ALI (Fig. 4B). ROC analysis showed that LINC00487 combined with hsa-miR-663b was a more reliable classifier for healthy persons and ALI patients compared with a single factor, with AUC of 0.840 (sensitivity = 75.0 %, specificity = 85.0 %) (Fig. 4C–E and I), and AUC of serum LINC00487/hsa-miR-663b to distinguish ALI patients from ARDS patients was 0.754 (sensitivity = 63.9 %, specificity = 88.9 %) and 0.706 (sensitivity = 86.1 %, specificity = 50.0 %), respectively (Fig. 4F–G and I). Their combination displayed a higher reliability, with an AUC of 0.822 (sensitivity = 75.0 %, specificity = 80.6 %) (Fig. 4H and I), indicating that the LINC00487/hsa-miR-663b axis is a classifier of ALI and ARDS and can effectively predict the progression of ALI. Moreover, the binary logistic regression analysis showed that for each unit increase in serum LINC00487, the risk of ALI patients developing ARDS increased by 45.4 % (P = 0.001); and for each unit increase in serum hsa-miR-663b, the risk of ALI patients developing ARDS was reduced by 32.0 % (P = 0.013) (Table 1), suggesting that both LINC00487 and hsa-miR-663b were independent factors affecting the progression of ALI.

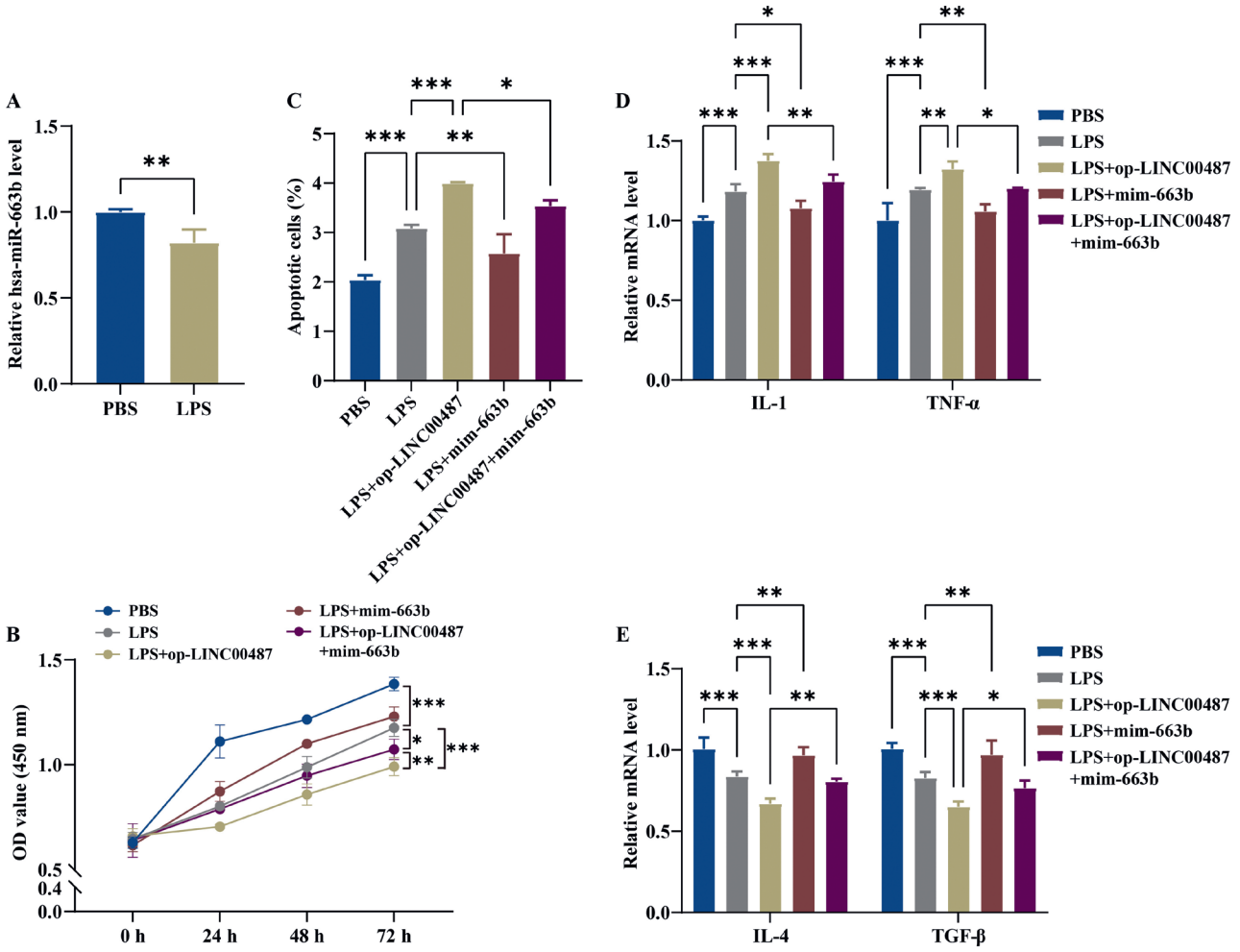
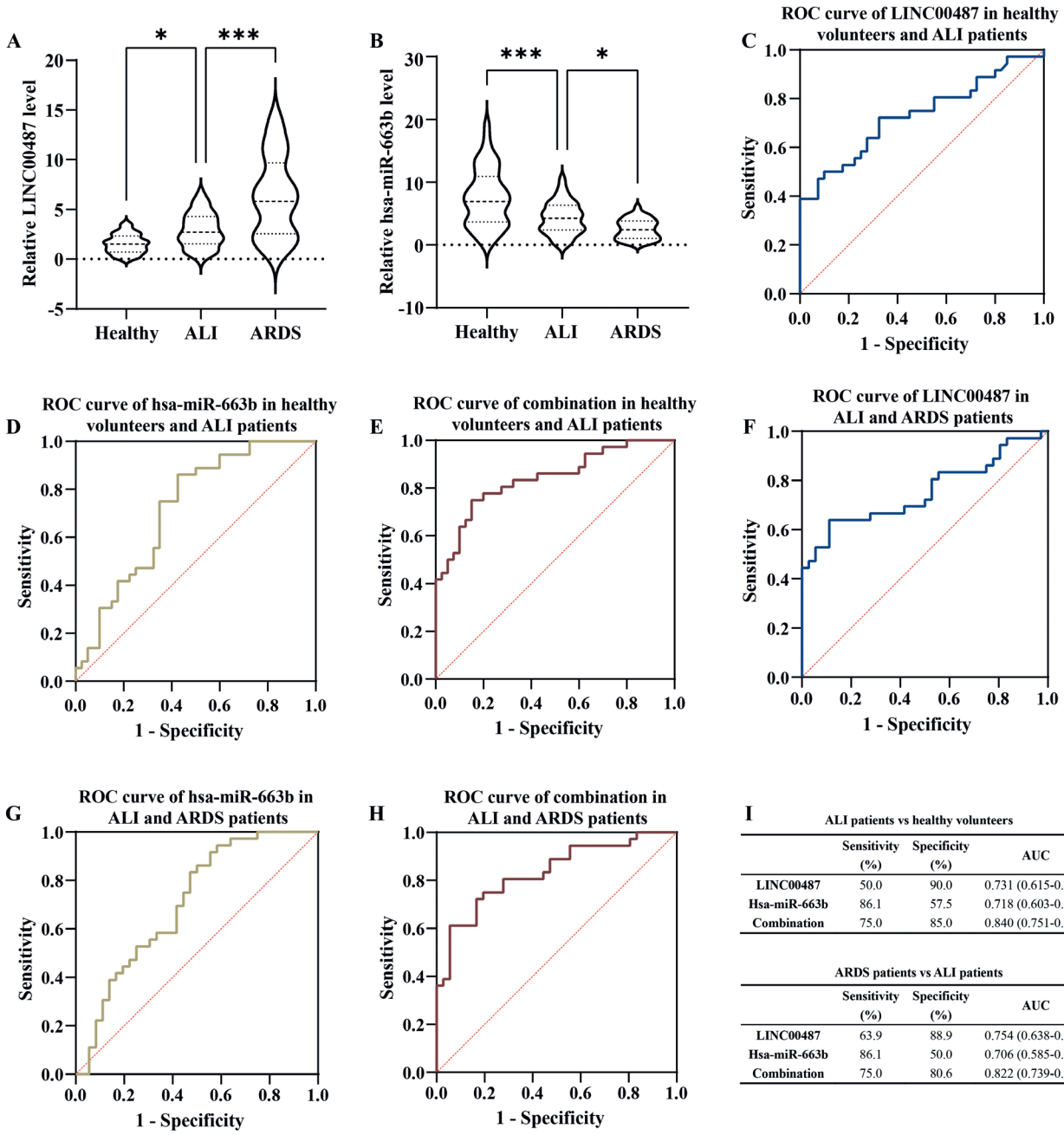


Fig. 3. Effect of hsa-miR-663b on the function of LINC00487. **(A)** Expression of hsa-miR-663b in HBEC3-KT cells with LPS treatment. Effects of hsa-miR-663b mimic on the proliferation inhibition **(B)**, elevated apoptosis rate **(C)** and inflammation **(D** and **E)**, induced by LINC00487 over-expression, in LPS-treated HBEC3-KT cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Discussion

ALI is a life-threatening disease with high morbidity and mortality. Many clinical diseases (such as pneumonia, tuberculosis, sepsis, etc.) will progress to ALI and even ARDS. Therefore, there is an urgent need to explore effective biomarkers for early prediction of ALI onset and monitoring its progression. In this study, by utilizing the publicly available datasets in the GEO database, we discovered a new lncRNA LINC00487 that might be related to the progress of ALI. LPS is a component of the cell wall of gram-negative bacteria and is composed of lipids and polysaccharides. As an endotoxin, LPS can cause a wide range of pathogenic effects when acting on other organisms or cells such as humans and animals, and it is also widely used to induce ALI mouse or cell models (Hou et al., 2021; Wang et al., 2023). Our data show that over-expression of LINC00487 aggravated the proliferation inhibition, inflammation and apoptosis of HBEC3-KT cells induced by LPS, while its silencing protected the cells from these deleterious effects. Through bioinformatic methods, hsa-miR-663b was predicted to be the key downstream target of LINC00487 that played a role in ALI, and our experiments also proved this. LINC00487 reduced the intracellular level of hsa-miR-663b by sponging it. Over-expression of hsa-miR-663b attenuated the damaging effect of LINC00487 on HBEC3-KT cells.

As expounded in the "Introduction", lncRNA and miRNA have been widely reported to be involved in the progression of ALI and are its potential markers. Our study showed that LINC00487 was up-regulated in LPS-induced HBEC3-KT cells, but hsa-miR-663b was under-expressed. Similarly, LINC00487 was highly expressed in the serum of ALI patients (vs healthy volunteers) and ARDS patients (vs ALI patients), and hsa-miR-663b was deficient. In addition, their combination had high accuracy in distinguishing healthy persons from ALI patients, as well as in distinguishing ALI patients from ARDS patients, with AUC of 0.840 and



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ALI patients vs healthy volunteers

	Sensitivity (%)	Specificity (%)	AUC
LINC00487	50.0	90.0	0.731 (0.615-0.847)
Hsa-miR-663b	86.1	57.5	0.718 (0.603-0.833)
Combination	75.0	85.0	0.840 (0.751-0.930)

ARDS patients vs ALI patients

	Sensitivity (%)	Specificity (%)	AUC
LINC00487	63.9	88.9	0.754 (0.638-0.869)
Hsa-miR-663b	86.1	50.0	0.706 (0.585-0.827)
Combination	75.0	80.6	0.822 (0.739-0.926)

Fig. 4. Clinical value of LINC00487 and hsa-miR-663b in ALI. **(A–B)** Serum level of LINC00487 and hsa-miR-663b. ROC curves of serum LINC00487, hsa-miR-663b and their combination in differentiating healthy volunteers from ALI patients **(C–E)** and in distinguishing ALI patients from ARDS patients **(F–H)**. **(I)** AUC, sensitivity and specificity values of the ROC curves. $^{*}P < 0.05$, $^{***}P < 0.001$.

0.822, respectively, suggesting that LINC00487 and hsa-miR-663b can be used as biomarkers of ALI and can effectively predict the development of ALI.

Existing studies have confirmed that lncRNAs have miRNA-binding sequences. LncRNAs often act as the sponge for miRNAs and reduce their intracellular levels through adsorption, thus regulating the progression of many human diseases, including ALI. Hong et al. (2021) revealed up-regulation of lncRNA SNHG14/Foxo3a and down-regulation of miR-223-3p in the lung tissues from LPS-induced mouse models. They have shown that, as a ceRNA, SNHG14 can bind to miR-223-3p to promote the expression of Foxo3a, which is involved in LPS-induced alveolar type II epithelial cell injury and ALI in mice through the regulation of autophagy. LncRNA XIST is enriched in monocyte-derived exosomes. XIST, transferred into HBE1 cells by these exosomes, can competitively inhibit the binding of miR-

Table 1. Binary logistic regression analysis

Factors	P value	OR	95% CI	
			Lower	Upper
LINC00487	0.001	1.454	1.157	1.827
Hsa-miR-663b	0.013	0.680	0.502	0.921
Age	0.549	1.019	0.958	1.084
Gender	0.246	2.041	0.611	6.815
Smoking history	0.710	1.261	0.371	4.285
Drinking history	0.378	1.731	0.511	5.869

Notes: OR – odds ratio; CI – confidence interval.

448-5p to HMGB2 mRNA, thereby up-regulating HMGB2 and ultimately leading to ALI in mice (Li et al., 2023b). The injury of alveolar epithelial cells can cause acute hypoxic respiratory insufficiency. LncRNA PFI can inhibit apoptosis of alveolar epithelial cells by regulating the miR-328-3p/Creb1 axis and alleviating LPS-induced ALI response (Li et al., 2023b). In our study, the dual luciferase assay demonstrated the interaction between LINC00487 and hsa-miR-663b, and LINC00487 negatively regulated the intracellular level of hsa-miR-663b. The increased level of hsa-miR-663b mitigated the ALI response of HBEC3-KT cells induced by LPS and over-expression of LINC00487. In the future, we will focus on the regulatory effect of hsa-miR-663b on downstream target genes to better investigate the pathogenesis of ALI.

The role of lncRNA LINC00487 in ALI has not been reported, but studies have shown that it is involved in the progression of other human diseases. The expression of LINC00487 was significantly elevated in all B-cell subsets from the serum of patients with primary Sjogren's syndrome (pSS). Furthermore, Inamo et al. (2020) suggest that LINC00487 is significantly associated with disease activity scores for all pSS B-cell subsets. According to Wang et al. (2022), the lncRNA-regulating epigenetic event signature (ELncSig), established by LINC00487 in combination with eight other lncRNAs including PRKCQ-AS1, LINC00877, etc., is an independent prognostic factor for diffuse large B-cell lymphoma, which includes four favourable prognostic factors, including LINC00487, and five adverse factors. There are more studies of hsa-miR-663b in human cancer. It can regulate the Ras/Raf signalling by down-regulating TNK1 and promote the development of colorectal cancer (Hong et al., 2020). Hsa-miR-663b is also significantly up-regulated in the plasma of patients with bladder cancer (BC), and the exosome hsa-miR-663b can target Ets2 repressor and promote the proliferation and epithelial-mesenchymal transformation of BC cells (Yin et al., 2020). Moreover, circulating hsa-miR-663b in the plasma is considered a potential novel biomarker of BC (Du et al., 2015). Studies have shown that over-expression of hsa-miR-663b can inhibit expression of inflammatory factors and reduce the apoptosis rate in nucleus pulposus cells induced by interleukin 1 (Li et al., 2023a). Furthermore, Liu et al. (2021a) indicate that hsa-miR-663b is central to the ceRNA network sequenced from human bronchial epithelial cells (16HBE) treated with and without LPS and plays a role in LPS-induced 16HBE autophagy, indicating the importance of hsa-miR-663b in ALI.

In conclusion, we revealed that LINC00487 and hsa-miR-663b were valuable biomarkers of ALI, and their combination can not only accurately diagnose the onset of ALI but also effectively detect the progression of ALI. Mechanistically, LINC00487 can participate in LPS-induced ALI response by negatively regulating hsa-miR-663b. Our study has certain reference significance for the treatment and diagnosis of ALI.

Author contributions

Conceptualization, X. Y., Z. X. and X. W.; Data curation, X. Z., Q. X. and J. Y.; Formal analysis, X. Z., Q. X. and J. Y.; Funding acquisition, X. W.; Investigation, X. Y. and X. Z.; Methodology, Z. X., Q. X. and J. Y.; Project administration, X. W.; Resources, X. Y. and X. Z.; Software, Q. X. and J. Y.; Supervision, X. W.; Validation, Q. X. and J.Y.; Visualization X. W.; Roles/Writing – original draft, Z. X.; Writing – review and editing, X. Y., X. W.

Data availability

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

References

Cui, Y., Wang, X., Lin, F. et al. (2022) MiR-29a-3p improves acute lung injury by reducing alveolar epithelial cell PANoptosis. *Aging Dis*. **13**, 899-909.

Du, M., Shi, D., Yuan, L. et al. (2015) Circulating miR-497 and miR-663b in plasma are potential novel biomarkers for bladder cancer. *Sci. Rep*. **5**, 10437.

Hong, J., Mo, S., Gong, F. et al. (2021) lncRNA-SNHG14 plays a role in acute lung injury induced by lipopolysaccharide through regulating autophagy via miR-223-3p/Foxo3a. *Mediators Inflamm*. **2021**, 7890288.

Hong, S., Yan, Z., Wang, H. et al. (2020) miR-663b promotes colorectal cancer progression by activating Ras/Raf signaling through downregulation of TNK1. *Hum. Cell* **33**, 104-115.

Hou, L., Zhang, J., Liu, Y. et al. (2021) MitoQ alleviates LPS-mediated acute lung injury through regulating Nrf2/Drp1 pathway. *Free Radic. Biol. Med.* **165**, 219-228.

Hu, Q., Zhang, S., Yang, Y. et al. (2022) Extracellular vesicles in the pathogenesis and treatment of acute lung injury. *Mil. Med. Res.* **9**, 61.

Inamo, J., Suzuki, K., Takeshita, M. et al. (2020) Identification of novel genes associated with dysregulation of B cells in patients with primary Sjogren's syndrome. *Arthritis Res. Ther.* **22**, 153.

Khemani, R. G., Smith, L., Lopez-Fernandez, Y. M. et al. (2019) Paediatric acute respiratory distress syndrome incidence and epidemiology (PARDIE): an international, observational study. *Lancet Respir. Med.* **7**, 115-128.

Li, Y. X., Ma, X. X., Zhao, C. L. et al. (2023a) The effect of microRNA-663b in the inhibition of interleukin-1-induced nucleus pulposus cell apoptosis and inflammatory response. *J. Physiol. Pharmacol.* **74**. https://doi.org/10.26402/jpp.2023.1.09

Li, Z., Jin, T., Yang, R. et al. (2023b) Long non-coding RNA PFI inhibits apoptosis of alveolar epithelial cells to alleviate lung injury via miR-328-3p/Creb1 axis. *Exp. Cell Res.* **430**, 113685.

Liu, J. Y., Jiang, Y. X., Zhang, M. Y. et al. (2021a) Comprehensive bioinformatics analysis of lipopolysaccharide-induced altered autophagy in acute lung injury and construction of underlying competing endogenous RNA regulatory mechanism. *Biomed Res. Int.* **2021**, 6831770.

Liu, X., Gao, C., Wang, Y. et al. (2021b) BMSC-derived exosomes ameliorate LPS-induced acute lung injury by miR-384-5p-controlled alveolar macrophage autophagy. *Oxid. Med. Cell. Longev*. **2021**, 9973457.

Long, M. E., Mallampalli, R. K., Horowitz, J. C. (2022) Pathogenesis of pneumonia and acute lung injury. *Clin. Sci. (Lond.)* **136**, 747-769.

Suresh, R., Kupfer, Y. Tessler, S. (2000) Acute respiratory distress syndrome. *N. Engl. J. Med.* **343**, 660-661.

Wang, X., Lu, Y., Liu, Z. et al. (2022) A 9-lncRNA signature for predicting prognosis and immune response in diffuse large B-cell lymphoma. *Front. Immunol.* **13**, 813031.

Wang, Y., Wang, Y., Ma, J. et al. (2023) YuPingFengSan ameliorates LPS-induced acute lung injury and gut barrier dysfunction in mice. *J. Ethnopharmacol.* **312**, 116452.

Wei, S., Liu, Q. (2019) Long noncoding RNA MALAT1 modulates sepsis-induced cardiac inflammation through the miR-150-5p/NF-κB axis. *Int. J. Clin. Exp. Pathol.* **12**, 3311-3319.

Yang, Q., Zhang, X., Luo, L. et al. (2023) Clinical application of serum NLRP3 on the diagnosis and prognosis of sepsis patients complicated with acute respiratory distress syndrome. *Front. Immunol.* **14**, 1205132.

Yin, X., Zheng, X., Liu, M. et al. (2020) Exosomal miR-663b targets Ets2-repressor factor to promote proliferation and the epithelial-mesenchymal transition of bladder cancer cells. *Cell Biol. Int.* **44**, 958-965.

Zhou, Y., Sun, L., Zhu, M. et al. (2022) Effects and early diagnostic value of lncRNA H19 on sepsis-induced acute lung injury. *Exp. Ther. Med.* **23**, 279.

Zhu, Y., Han, Q., Wang, L. et al. (2023) Jinhua Qinggan granules attenuates acute lung injury by promotion of neutrophil apoptosis and inhibition of TLR4/MyD88/NF-κB pathway. *J. Ethnopharmacol.* **301**, 115763.