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RESEARCH ARTICLE

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Effects of dexmedetomidine on the expression profile of tsRNAs in LPS-induced acute lung injury

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Abstract

Background: Acute lung injury (ALI) is characterized by impaired alveolar function and excessive inflammation, which is commonly seen in clinical anesthesia and intensive care units. tRNA-derived small RNA (tsRNA) is a non-coding RNA that can be used as a potential disease diagnostic biomarker. The connection between ALI and tsRNA remains unknown. We aimed to explore the possible regulatory functions and mechanisms of tsRNAs in ALI treated with DEX.

Methods: Firstly, we established the ALI model by LPS injection and explored the effect of dexmedetomidine (DEX) treatment on lung damage. Then, the lung tissues were obtained from the LPS and LPS + DEX group for small RNA sequencing.

Results: We proved that DEX could ameliorate pulmonary injury, and decreased inflammation, pulmonary edema, and ferroptosis (MDA down-regulation and GPX4 up-regulation) in ALI. Furthermore, in the tsRNA expression profile, the top 10 down-regulated tsRNAs were tsRNA-1018, tsRNA-3045b, tsRNA-5021a, tsRNA-1020, tsRNA-5002b, tsRNA-3045b, tsRNA-1026, tsRNA-5004a, tsRNA-5005b and tsRNA-1009, and the top 10 up-regulated tsRNAs were tsRNA-3025b, tsRNA-3025a, tsRNA-5016b, tsRNA-3042b, tsRNA-3029b, tsRNA-3028b, tsRNA-5006a, tsRNA-3027b, tsRNA-3027a, and tsRNA-5009b. The enrichment analysis of GO terms and KEGG pathways pointed that target genes of DE-tsRNAs were mainly enriched in regulation of transcription-associated GO terms, NF-kappa B signaling pathway, MAPK signaling pathway, and PI3K-Akt signaling pathway. The RT-qPCR results of tsRNA-1020 and tsRNA-1018 were in accordance with small RNA sequencing data.

Conclusion: DEX affected the abnormal expression of tsRNAs in ALI. These aberrantly expressed tsRNAs and enriched physiological processes provide a scientific basis for the diagnosis and treatment of ALI.

KEYWORDS
acute lung injury, dexmedetomidine, expression profile, sequencing, tRNA-derived small RNAs

Yue Lin and Junying Cai contributed equally as first authors.

1 | INTRODUCTION

Acute lung injury (ALI) is a disease in which the lung endothelial and epithelial barriers are destroyed by acute inflammation. Due to the high morbidity, the mortality rate of ALI patients is also high.[1] It is characterized by excessive inflammation, which eventually leads to accumulation of inflammatory factors, pulmonary edema, and alveolar epithelial damage.[2,3] Recently, the understanding of the pathogenesis of ALI has made good progress. However, practice has demonstrated that conventional drug treatments cannot effectively reduce the mortality of ALI patients.[4] Therefore, clarifying the pathogenesis of ALI and developing molecular targeted drugs are of great significance for the treatment of the disease.

Dexmedetomidine (DEX) is a sedative and anti-analgesic drug, which is widely used in intensive care units.[5] The reason why DEX received attention is that it has been clinically shown to reduce ALI. A previous study showed that DEX might reduce the damage of ALI via NF-κB and PI3K pathways.[6] DEX attenuated the aggravation of sepsis-stimulated ALI via down-regulation of the RAGE pathway.[7] At present, studies have reported that ALI patients were mostly accompanied by ferroptosis.[8] However, whether DEX alleviates ferroptosis in ALI remains unclear, and the molecular mechanism involved in this process needs to be further elucidated.

Transfer RNA-derived small RNA (tsRNA) is a non-coding RNA that has the ability to modulate a variety of physiological processes.[9] Recent studies revealed that tsRNAs, similar to miRNAs, can regulate the stability of mRNA through a binding mechanism.[10,11] It was previously reported that DEX targeted NLRP3 through miR-381 to reduce lipopolysaccharide (LPS)-induced ALI.[12] However, whether DEX treats ALI by tsRNA binding to target genes has never been reported. In the current research, we firstly explored the tsRNAs associated with ALI. Moreover, we also explored whether and which tsRNAs are necessary in the process of DEX attenuating ALI. Our research will provide a new scientific basis for the molecular mechanism of DEX in the treatment of ALI and provide a theoretical basis for the clinical use of DEX in the treatment of ALI.

2 | MATERIALS AND METHODS

2.1 | Animals

C57BL/6 mice (male, 6–8 weeks of age, 20–25 g) were purchased from the Cloud-Clone Animal Inc. (Wuhan, China) and were raised in cages with free food and water, and used after 2 weeks of isolation and domestication. All procedures involving animals were approved by the Second Affiliated Hospital of Nanchang University Animal Ethics Community. All operations were performed under sodium pentobarbital anesthesia.

2.2 | Murine model of LPS-induced ALI

The mice were randomly divided into 3 groups (n = 5 mice each): the control group injected with normal saline, the LPS group injected with 5 mg/kg of LPS, and before LPS administration 1 h the LPS + DEX group injected with DEX (25 μg/kg).[12] All animals were injected in the abdomen. The lung injury was assessed 6 h after LPS administration.

2.3 | Lung wet/dry (W/D) weight measurement

The W/D ratio was used to evaluate the severity of pulmonary edema. Briefly, after mice sacrifice, lung tissues were immediately collected and weighed. The weight of the removed lung tissues is the wet weight (W). Then, we put the wet lung tissues in a 60°C oven for 48-h to obtain a dry weight (D). Finally, we calculated the W/D ratio.

2.4 | Hematoxylin–eosin (HE) staining

Fresh mouse lung tissues were fixed in 10% formalin for 24 h. Then, lung tissues were dehydrated, embedded in paraffin, cut into 3-μm slices, and stained with hematoxylin and eosin. Finally, we analyzed the pathological changes of lung tissues.

2.5 | Evaluation of malondialdehyde (MDA) level

The malondialdehyde (MDA) level in lung tissues and cells was assessed using the Lipid Peroxidation (MDA) Assay Kit (Sigma, Cat #: MAK085), according to the manufacturer's instructions.

2.6 | Immunohistochemistry

Immunohistochemistry for glutathione peroxidase 4 (GPX4) was conducted. The sections were blocked with 8% goat serum in PBS and then incubated with anti-GPX4 antibody (1:1000; Santa Cruz, Cat#: sc-166,570) at 4°C for 12 h. Thereafter, the sections were incubated with anti-mouse HRP reagent (Sigma-Aldrich, Cat#: A-9044) at room temperature for 1 h. Finally, the sections were examined under a light microscope and Nikon Photo-Imaging system (H550L, Tokyo, Japan).

2.7 | RNA isolation, small RNA libraries construction, and sequencing

The total RNA was obtained from the LPS group and the LPS + DEX group of mice lung (n = 3 for each group) by the RNeasy Mini Kit (Qiagen, Hilden, Germany). The quality of RNA was detected by the 1% agarose gel electrophoresis. The concentration and integrity of total RNA were measured by the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). Six independent small RNA libraries of the LPS and the LPS + DEX mice lungs were constructed by the manufacturer's protocol. Following passing

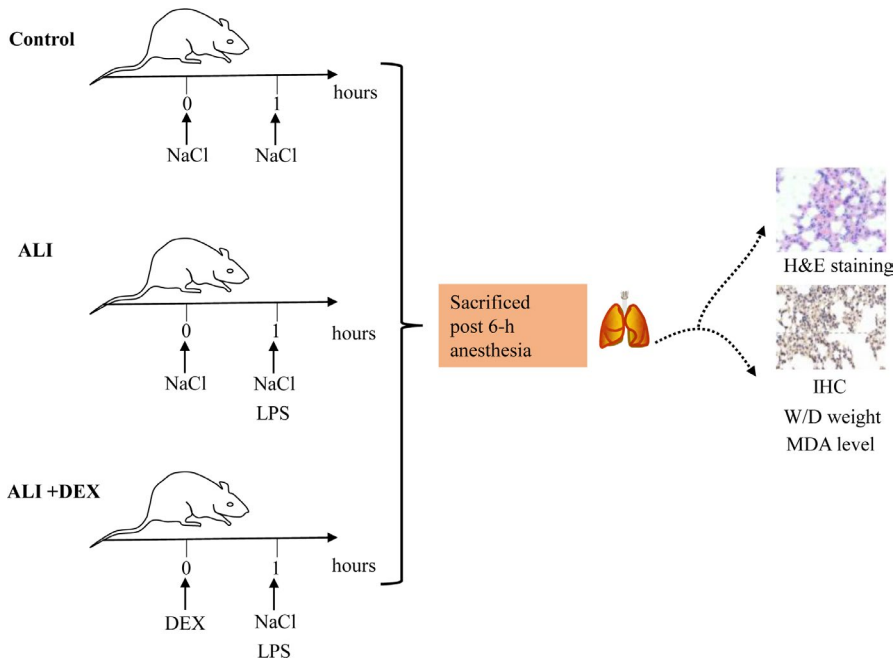

FIGURE 1 Construction of an animal model of LPS-induced ALI. The mice were divided randomly into 3 groups ($n = 5$ mice each): the control group, the LPS group, and the LPS + DEX group

the quality control tests, the Multiplex Small RNA Library Prep Set for Illumina (NEB, MA, USA) was leveraged to establish the library. Briefly, the rRNA was removed and the remaining RNA was cut into small pieces. RNase H-reverse transcriptase (NEB, MA, USA) was used to synthesize the first-strand cDNA and the end repair was conducted, and then, the fragments were sorted and PCR was performed to amplify the cDNA. An Illumina Hiseq2500 platform (Illumina, San Diego, California) was used for the sequencing of library preparations.

2.8 | Identification of DE-tsRNA

The raw reads were processed by Fast-QC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The BWA algorithm was used to mapping the mice genome, and miRBase Version 21 (http://www.mirbase.org/) was leveraged to annotate the tsRNA. The DESeq 2.0 algorithm was utilized to analyze the differentially expressed tsRNAs (DE-tsRNAs) between the LPS and the LPS + DEX group mice lung with |fold change| >1.5 and p-value <0.05 as the inclusion criteria.

2.9 | Target prediction and annotation

The targeted genes of tsRNAs were predicted by miRanda (http://www.microrna.org/microrna/home.do) and RNAHybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/), and miRanda predicted the target genes of DE-tsRNAs with Score ≥ 150 and Energy < −20 as the inclusion criteria, whereas inclusion criteria of target genes were Energy < −25 in RNAhybrid. Ultimately, the target genes were determined by the intersection of the two databases. GO (http://www.geneontology.org) enrichment analysis was performed to annotate the target genes from the respect of cellular component, molecular function, and biological process. KEGG (http://www.genome.jp/kegg) database was used to analyze the pathway of target genes.

2.10 | Real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR)

The top 5 differentially expressed tsRNAs from array analysis were further detected by qRT-PCR in lung tissues from 3 pairs of mice. The total RNAs were extracted using Trizol reagent (Invitrogen, CA, USA) and subjected to obtain cDNA by PrimeScript^{TM} RT reagent kit (Takara, Dalian, China). RT-qPCR analysis was performed using Fast SYBR Green master mix (Applied Biosystems, Life Technologies, New York, NY, USA). U6 was utilized as an internal control. The relative tsRNAs expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were presented in Table S1.

2.11 | Construction of the tsRNA-mRNA network

To display the correlation between the validated tsRNA and mRNA, a network of tsRNA-mRNA was established. Cytoscape software 3.6.1 (https://cytoscape.org/) was utilized to draw the tsRNA-target genes interaction network.

2.12 | Statistical analysis

All data were analyzed using GraphPad Prism 8.0. (La Jolla, CA, USA). Student's t test was used for comparing the difference between the two groups. Data are shown as the mean ± SD. Differences were deemed statistically significant at $p < 0.05$.

3 | RESULTS

3.1 | DEX treatment alleviated ferroptosis

To investigate the effect of DEX on ALI, we injected DEX in mice 1 h before LPS administration (Figure 1). For the wet to dry ratio, we found that it was significantly increased in the LPS group, while decreased significantly after DEX treatment (Figure 2A). Moreover, as shown in Figure 2B, the normal lung tissue structure in the LPS group was destroyed, and the alveoli, alveolar ducts, and bronchi were diffusely damaged. However, the addition of DEX alleviated these lung injuries (Figure 2B). Furthermore, DEX reversed the effects of LPS-induced upregulation of MDA (Figure 2C) and down-regulation of GPX4 (Figure 2D) expression in the lungs. These results demonstrated the protecting role of DEX in ALI by ferroptosis.

3.2 | Summary of the tsRNA expression profiles in lung tissues

To explore abnormally expressed tsRNAs between LPS and LPS + DEX groups in lung tissues, small RNA sequencing was performed. As depicted in Table 1, after removing low-quality reads from raw reads, 51,762,104 and 56,654,855 clean reads were, respectively, obtained from lung tissues in the LPS group and the LPS + DEX group, and an average of 44,351,441 (85.7% mapped rate) and 46,175,156 (81.5% mapped rate) clean reads were respectively mapped to the mice genome. Length analysis indicated that the most sequences were 15–31 nt long (Figure S1), which was consistent with the typical characteristics of Dicer-processed products.[13]

3.3 | Differential tsRNAs expression analysis

According to transcriptional sequencing, we obtained the expression profile of tsRNA of which the top 10 down-regulated tsRNAs were tsRNA-1018, tsRNA-3045b, tsRNA-5021a, tsRNA-1020, tsRNA-5002b, tsRNA-3045b, tsRNA-1026, tsRNA-5004a, tsRNA-5005b and tsRNA-1009, and the top 10 up-regulated tsRNAs were tsRNA-3025b, tsRNA-3025a, tsRNA-5016b, tsRNA-3042b, tsRNA-3029b, tsRNA-3028b, tsRNA-5006a, tsRNA-3027b, tsRNA-3027a, and tsRNA-5009b. Moreover, according to the criteria with |fold change| >2 and p-value <0.05, a heatmap was executed and distinct patterns of tsRNA expression were observed in LPS and LPS + DEX groups. Cluster analysis revealed distinct profiles of tsRNA expression between LPS and LPS + DEX groups (Figure 3A). There were 4 down-regulated tsRNAs (tsRNA-1018, tsRNA-3045b, tsRNA-5021a, and tsRNA-1020) and 1 up-regulated tsRNA (tsRNA-3025b) in the LPS + DEX group relative to the LPS group. A volcano plot also showed some tsRNAs were down-regulated by more than 1.5-fold in the LPS + DEX group relative to the LPS group (Figure 3B).

3.4 | Functional and pathway analysis of the predicted targeted genes of DE-tsRNAs

To further recognize the possible functions and mechanisms of the DE-tsRNAs with DEX treatment, the function analysis was performed. A total of 242 target genes for the DE-tsRNAs were obtained through the intersection of the RNAHybrid and miRanda algorithms (Figure 4A). GO analysis showed some critical biological processes for DE-tsRNAs enrichment, such as "regulation of

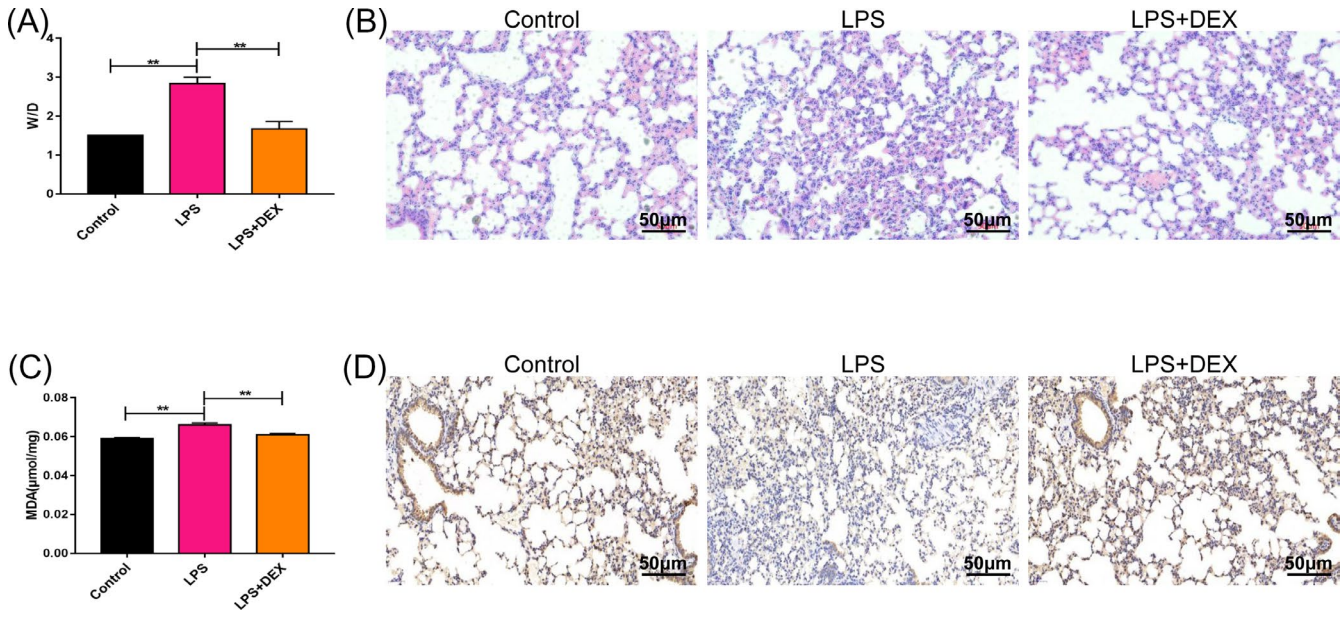

FIGURE 2 Effect of DEX on LPS-induced ALI. (A) The lung wet/dry ratio of different groups. (B) HE staining of lung tissue sections in each group (Scale bar = 50 μm). (C) The MDA level detected via the MDA Assay Kit. (D) In vivo validation of the protein expression of GPX4 using immunohistochemical staining (Scale bar = 50 μm). *$p < 0.05$, **$p < 0.01$, $n = 5$

TABLE 1 Summary of the sequencing reads alignment to the reference genome

Samples	Clean reads	Mapped reads	Mapped rate (%)	Clean bases	Mapped bases	Mapped rate (%)
LPS1	47458017	40781506	85.9	7118541146	1058793953	14.9%
LPS2	54838769	47287235	86.2	8225630742	1192479857	14.5%
LPS3	52989527	44985583	84.9	7948254610	1150331203	14.5%
LPS-DEX1	64235032	54670806	85.1	9635036164	1442606339	15.0%
LPS-DEX2	59324942	44960632	75.8	8898539538	1196272276	13.4%
LPS-DEX3	46404592	38894029	83.8	6960529785	1032281137	14.8%

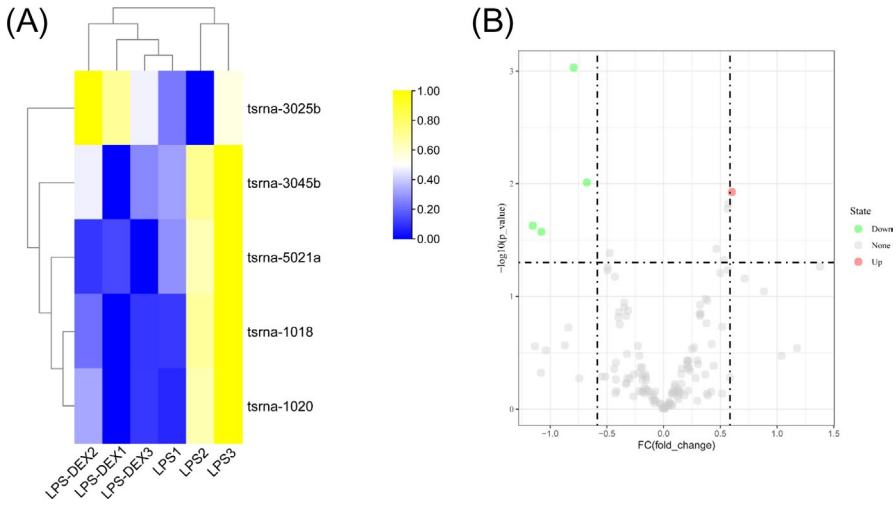

FIGURE 3 DE-tsRNAs in LPS + DEX groups compared to LPS groups. (A) Hierarchical clustering with RPKM of DE-tsRNAs in LPS and LPS + DEX groups. Yellow represented up-regulated DE-tsRNAs, and blue showed down-regulated DE-tsRNAs in LPS + DEX groups. (B) Volcano plot showed DE-tsRNAs between LPS and LPS + DEX groups. The green dots indicated the down-regulated DE-tsRNAs, and red dots indicated the up-regulated DE-tsRNAs

transcription, DNA-templated," "actin filament organization," "positive regulation of transcription from RNA polymerase II promoter," and "negative regulation of immune response" (Figure 4B). Furthermore, the target genes of tsRNAs were mainly associated with "MAPK signaling pathway," "NF-kappa B signaling pathway," "mTOR signaling pathway," and "PI3K-Akt signaling pathway" (Figure 4C).

3.5 | Validation of tsRNAs

To validate our tsRNAs sequencing, we selected 5 DE-tsRNAs from the above tsRNAs, including four down-regulated tsRNAs (tsRNA-1018, tsRNA-3045b, tsRNA-5021a, and tsRNA-1020) and one up-regulated tsRNA (tsRNA-3025b) to perform qRT-PCR validation. The results showed that tsRNA-1018 and tsRNA-1020 were down-regulated, similar to the expression trend in the tsRNAs transcriptome (Figure 5).

3.6 | Construction of the tsRNA-mRNA pathway

For the specific mRNA targets of DE-tsRNAs, we also compared the five tsRNA-mRNA targets verified by qRT-PCR with the mRNA targets detected by mRNA sequencing to further strengthen the results (Figure 6). tsRNA-1018, tsRNA-3045b, tsRNA-5021a, and tsRNA-1020 regulated the key target gene through the PI3K-Akt signaling pathway and MAPK signaling pathway. tsRNA-3025b targeted Plcb1 (Phospholipase C Beta 1) and participated in inflammatory mediator regulation of TRP channels.

4 | DISCUSSION

Clinically, DEX is used as medicine to reduce anxiety, sedation, and pain. In recent years, studies have reported that DEX reduces the symptoms of ALI.[14] Genomics technologies have been extensively used to illustrate the relationship between adaptive phenotype and genetic information in plants, animals, and humans.[15,16] However, there is little known about the role of the tsRNAs in ALI treated with DEX. In our study, LPS-induced ALI in mice manifested by destroyed pulmonary edema, thickened alveolar septa, and alveolar hemorrhage, and LPS increased the content of MDA in lung tissues and decreased the expression of GPX4, whereas these ALI symptoms were effectively alleviated with DEX treatment. Through small RNA sequencing and analysis, we found 5 DE-tsRNAs in mice treated with DEX. These findings indicated that DE-tsRNAs induced by DEX hold promise as potential targets for applying for ALI treatment.

ALI is a common serious disease clinically, and its potential therapeutic mechanism and pathogenesis are not yet known.[5] Generally, LPS-induced ALI is pathologically characterized by lung tissue damage, pulmonary inflammation and edema, accompanied by ferroptosis.[17] More and more studies have reported that DEX has a protective effect on the lungs. For example, DEX pretreatment

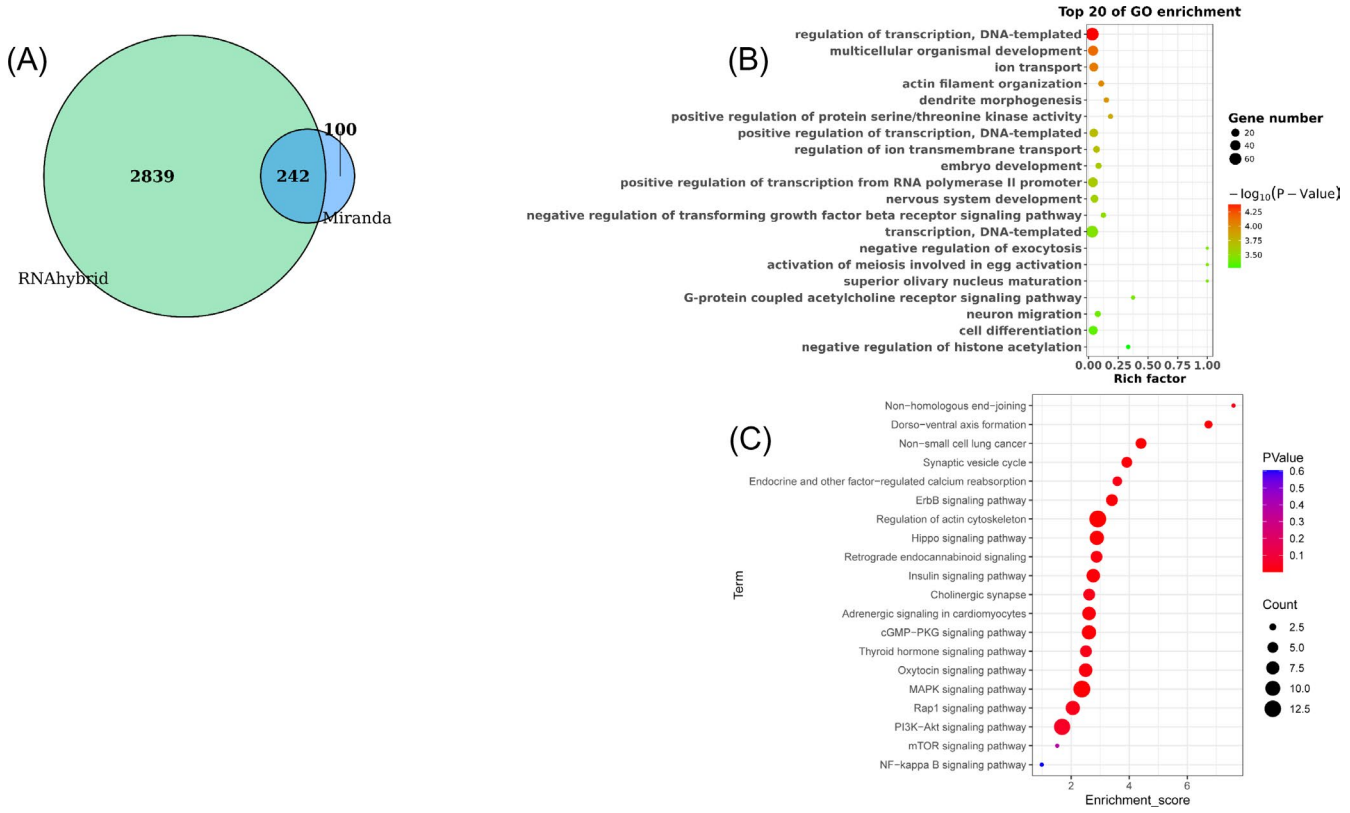

FIGURE 4 Target genes prediction and functional enrichment analyses of the DE-tsRNAs. (A) Venn diagrams of the number of target genes for DE-tsRNAs in two groups using RNAhybrid and Miranda algorithms. (B) GO enrichment analysis of predicted target genes of DE-tsRNAs. (C) KEGG-enriched terms of predicted target genes of DE-tsRNAs between LPS and LPS + DEX groups

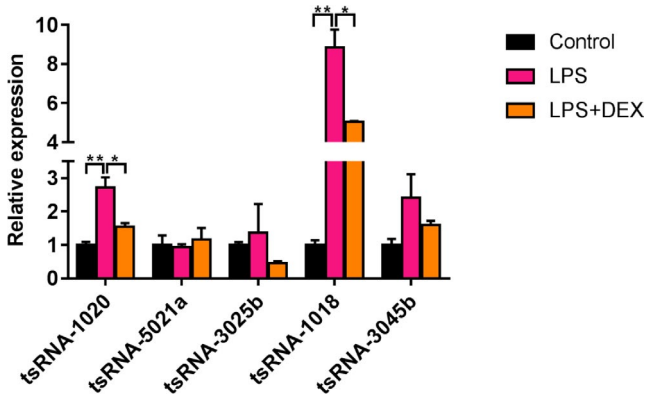

FIGURE 5 Validation of candidate DE-tsRNAs. The expression level of candidate DE-tsRNAs using RT-qPCR. The values are expressed as mean ± SD ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$

could attenuate lung tissue damage caused by LPS.[18] DEX improved LPS-induced ALI through NF-κB signaling pathway.[19] Similarly, the results of this study indicated that DEX alleviated the severity of pulmonary edema, improved pathological lung changes, and weakened the inflammatory response. In addition, DEX alleviated sepsis induced myocardial ferroptosis, including increasing the expression of GPX4.[20] Consistently, our results also found that DEX effectively alleviated ferroptosis symptoms, including decreased MDA and increased GPX4. These results confirmed that DEX has a therapeutic effect on LPS-induced ALI in rats.

tsRNAs are an abundant class of small non-coding RNAs, which play a variety of roles in different physiological processes.[10,21] There is growing evidence that tsRNAs can affect gene expression at the post-transcriptional level by targeting 3'-UTR, showing a mechanism similar to microRNA (miRNA).[22,23] A previous study showed approximately 263 DE-tsRNAs in breast cancer, whereas most of them were decreased.[24] The ts-46 and ts-47 as tsRNAs that were strongly down-regulated in lung cancer.[25] Analogously, four tsRNAs (tsRNA-1018, tsRNA-3045b, tsRNA-5021a, and tsRNA-1020) were down-regulated and one tsRNA (tsRNA-3025b) was up-regulated in the LPS + DEX group compared to the LPS group in our research. The predicted target genes of these tsRNAs were associated with the PI3K-Akt signaling pathway. It is reported that DEX may protect LPS-induced ALI mice likely through the TLR4/NFκB and PI3K/Akt/mTOR pathways.[6] Similarly, Shi and her colleagues demonstrated heme oxygenase-1 (HO-1) protected the lung from oxidative damage through the PI3K/Akt pathway.[26] Additionally, DEX administration significantly increased HO-1 expression.[5] Moreover, MAPK signaling pathway and NF-kappa B signaling pathway are involved in inflammatory responses that regulate the expression of both pro- and anti-inflammatory mediators.[27,28] Generally, ALI patients are associated with elevated levels of proinflammatory factors. Importantly, the target genes of the DE-tsRNAs in our study are concentrated in pathways related to inflammatory factors, such as the MAPK signaling pathway and NF-kappa B signaling pathway. Given that the above report and our

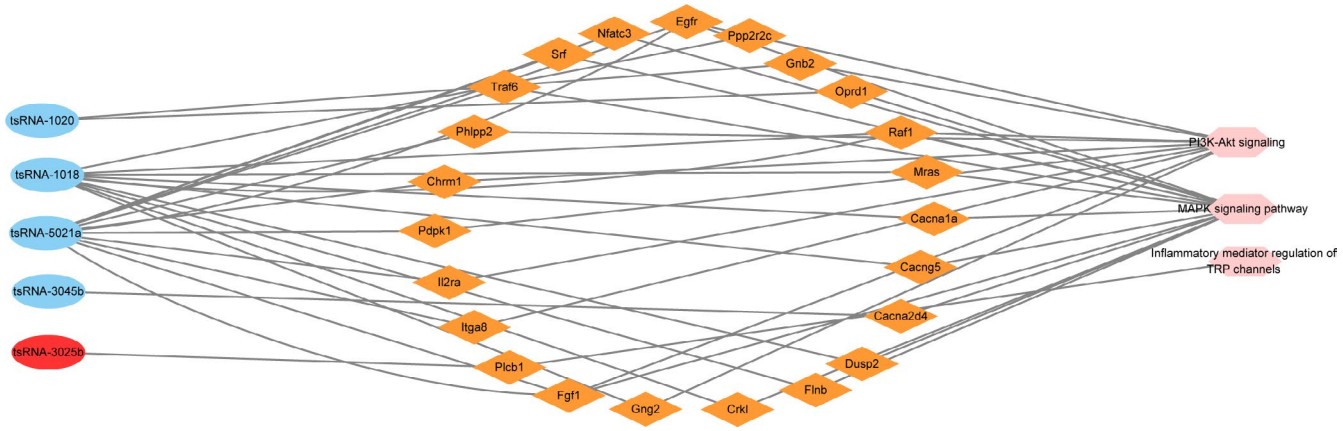

FIGURE 6 The interaction diagram of tsRNAs-mRNAs pathways. The ellipse indicated DE-tsRNAs, the diamond represented target genes, and the polygon showed pathways

study, tsRNAs may be responsible for DEX treating ALI via a variety of regulating signaling pathways.

5 | CONCLUSION

In summary, this study conducted tsRNAs profiling in ALI with DEX treatment. DEX can reduce the pathological features of ALI and ferroptosis. PI3K-Akt signaling pathway, MAPK signaling pathway, and NF-kappa B signaling pathway were highlighted by DE-tsRNAs and mRNAs in ALI, supporting the potential acceptance of ALI for DEX treatment. Further researches will be needed to clarify the complex mechanism of these tsRNAs and their related pathways for better understanding the therapeutic mechanism of DEX.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Jun Lu contributed to study concept and design. Yue Lin and Junying Cai performed the experiments, analyzed the data, and wrote the article. Dan Huang, Bin Zhou, Zhenzhong Luo, and Shuchun Yu contributed to the experimental work. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All procedures involving animals were approved by the Second Affiliated Hospital of Nanchang University.

DATA AVAILABILITY STATEMENT

The datasets supporting our findings are presented in the article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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