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Original Research

microRNA-23a-5p acts as a potential biomarker for sepsis-induced acute respiratory distress syndrome in early stage

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Abstract: Sepsis is a significant cause of morbidity and mortality worldwide. Acute respiratory distress syndrome (ARDS) is the most common and serious complication of sepsis, which presents with rapid and progressive acute onset respiratory failure. The microRNA-23a-5p, as a kind of circulating microRNA (miRNA), is considered to be a candidate biomarker for cardiovascular diseases. However, correlation between ARDS and miR-23a-5p is also elusive. This study aims to investigate the role of miR-23a-5p as the biomarkers for ARDS. In this study, ARDS was induced by intraperitoneally injected with LPS of Sprague-Dawley rats and serum and lung tissues were collected. The NR8383 macrophages were stimulated with LPS. TNF-α, IL-1β, and miR-23a-5p levels in serum, lung tissues and NR8383 were determined using SYBR-based miRNA quantitative real-time polymerase chain reactions (qRT-PCRs). The results indicated that serum miR-23a-5p was increased by 7 fold, 4 fold and 2 fold at 3 h, 6h, and 12h after injection of LPS, respectively. While the miR-23a-5p in NR8383 was elevated by 3.5 fold, 3 fold, 2.5 fold and 5 fold, at 3 h, 6h, 12h and 24h after stimulated with LPS, respectively. In conclusion, the miR-23a-5p might be employed as the potential biomarkers for ARDS in early stage.

Key words: microRNA-23a-5p, acute respiratory distress syndrome, biomarker, sepsis, LPS.

Introduction

Acute respiratory distress syndrome (ARDS) is defined as a rapidly progressive acute onset respiratory failure. ARDS is the most common and serious complication of sepsis, which presents with rapid and progressive acute onset respiratory failure, and is characterized by broad clinical presentations. Meanwhile, the ARDS is always caused by a variety of insults, such as bacterial or viral pneumonia, non-pulmonary sepsis, major trauma, amniotic fluid embolism, transfusions, aspiration of gastric contents, drug reactions *and ect.* ARDS is associated with world-wide high morbidity and mortality in clinical (1,2). The rapid diagnosis of ARDS is critical and significant for the clinicians to risk stratify the patients, and also guarantees the immediate appropriate treatment. Therefore, exploring novel biomarkers for ARDS is very necessary for clinicians (3-7).

MicroRNAs are endogenous, 20 to 25 nucleotide long non-coding RNAs that function as post transcriptional regulators of gene expression. The post transcriptional regulator function of microRNA was performed by specifically interacting with certain mRNAs and binding with the 3'UTR of the target mRNA, and then inducing their degradation or repressing their translation (8). According to the latest miRBase database 21, 1881 precursors and 2588 mature miRNAs have been identified in humans. Furthermore, more than 30% of human protein-encoding genes seem to be regulated by specific miRNA (9). As the currently known about the role of miRNAs, which participate in a variety of essential biological processes, such as cell proliferation, cell differentiation, development, cell apoptosis and autophagy (10-13). Previous studies have been found that the aberrant expression of miRNAs contributes to various diseases, such as tumor, cardiovascular disease, autoimmune disease, *and ect.* (14-17). From the year of 2007 to nowadays (18), considerable reports have indicated that miRNAs are in a consistent, stable and reproducible manner in the circulation. Therefore, the miRNAs may be promising as the potential and non-invasive diagnostic markers in some diseases (19-21).

The previous studies showed that the tissue specific microRNAs can be released into the circulation from the injured organs, therefore, exploring candidate microRNA as biomarkers for specific diseases is continuous. In the previous studies, miR-23a-5p has been considered to be the candidate biomarker for some diseases, such as traumatic injury (22), schizophrenia and schizoaffective disorder (23), and orofacial inflammatory pain (24), *and ect*. Furthermore, many microRNAs, such as miR-146a, miR-125b, miR-344, miR-346, miR-99a, miR-127, miR-128b, miR-135b, and miR-30a/b, have been found to be consistently elevated in circulation of acute lung injury (ALI) or ARDS (25-27). But the correlation between ARDS and miR-23a-5p has not been fully reported. Furthermore, the serum yields slightly higher miRNA and the majority of archived samples are stored in form of serum. Therefore, the about backgrounds marking it interesting to determine whether miR-23a-

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5p in serum can be used as the biomarkers for ARDS.

Materials and Methods

Grouping and generation of ARDS model in rats

The male Sprague-Dawley rats aged from 6 to 8 weeks, weighing from 250 to 300 g, were purchased from Guangdong medical laboratory animal center (Guangzhou, China). Endotoxemia was induced by administration of bacterial LPS from Escherichia coli 055: B5 (Sigma, USA), which was dissolved in sterile saline (1 mg/ml), via intraperitoneal injection (10 mg/kg). Twenty-four SD rats were randomly divided into four groups (6 rats per group), including model groups of LPS for 3h, LPS for 6h, LPS for 12h. The treatment of LPS could induce the lung injury, and trigger the expression of inflammatory cytokines. All of the rats were survival after injecting the LPS in this study. In addition, the normal control group received an equal volume of sterile saline (2.5-3.0 ml/body), without LPS (group N). All the procedures were approved by the Institutional Animal Care and Use Committee at the Southern Medical University, Guangzhou, China.

Criteria of ARDS model

Criteria for successful model of ARDS for lung tissue included the presence of diffuse alveolar damage (DAD), qualitatively assessed (yes/positive result or no/negative result) as the followings: ① alveolar type I cell necrosis, ②intra-alveolar edema, ③alveolar type II cell (cuboidal cells) proliferation progressively covering the denuded alveolar-capillary membrane, ④interstitial proliferation of fibroblasts and myofibroblasts, ⑤organizing interstitial fibrosis (28,29).

General observation of the rats

Breathing rates, mental state and response to stimulations from surroundings were compared between the model groups and the normal control groups.

Serum and tissue sampling and RNA isolation

SD rats were anesthetized with the chloral hydrate (7%, 0.5 ml/100g, i.p.) and killed at different time-points after LPS administration (3, 6, and 12 h, respectively). At the indicated time, the blood samples were collected by cardiac puncture for blood gas analysis, inflammatory cytokines analysis. For serum collection, whole blood samples were placed at room temperature for 1 h. After that, the samples were centrifuged at 3000 r/min for 20 min at 4 °C and the supernatant (serum) was transferred to RNase/DNase-free tubes and stored at -80 °C until RNA isolation. The total RNA was isolated from the serum using a miRNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Briefly, 400 µl of serum was mixed with 2 ml of QIAzol Lysis Reagent and 400 µl chloroform. The samples were centrifuged and the upper aqueous phase were transferred to collection tubes and mixed with 975 µl 100 % ethanol. The mixture was applied to RNeasy Minispin columns and purified according to the protocol. Total RNA was eluted using 30 µl of RNAse-free water. Samples were stored at -80°C. Lung tissues from ARDS and normal control rats were immediately flash frozen in liquid nitrogen, and stored at -80 °C until RNA isolation using a RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's instruction. For paraffin sections, lung tissue specimens were post-fixed in 4% paraformaldehyde overnight and then embedded in paraffin.

Lung wet-to-dry (W/D) weight ratio

The wet (W) and dry (D) weights (70 °C for 24 h in an electric air blast drier) of the middle lobe of right lung were measured. The lung W/D weight ratio was calculated by dividing the wet weight by the dry weight.

Histological analysis of lung tissues

After embedded and fixed in paraffin, the lung tissues were sectioned into 4 µm thickness, and stained with hematoxylin and eosin (HE) staining. The sections were scored as previously reported. The histological scoring parameters included edema of the alveoli, edema of the alveolar mesenchyme, intra-alveolar cell infiltration, alveolar hemorrhage, and atelectasis (30).

Cell culture and treatment

The NR8383 AM cell line (The cell bank, Chinese academy of sciences) as grown in plastic tissue culture flasks in Ham's F-12 medium containing 15% fetal bovine serum, 100 µg/ml penicillin and 100 U/ml streptomycin sulfate. The AMs were cultured at 37 °C in an atmosphere of 5 % CO2 in air. The media was routinely changed twice weekly. Cells were seeded to 12-well tissue culture plates at a density of 1×10^5 cells/well. Cell viability before and after each experiment were evaluated by using the trypan blue assay. The viability was consistently greater than 90% in all detected samples after treatment. The experimental design included the following AM treatment groups: 1) Normal control (no LPS); 2) LPS stimulation for 3 h; 3) LPS stimulation for 6 h; 4) LPS stimulation for 12 h; 5) LPS stimulation for 24 h. For cell stimulation, LPS was added to the medium at a final concentration of 1 µg/ml, and the cell growth condition and cell morphological changes of the cells were observed in each group time. Cells were harvested and total RNAs were 12-well tissue culture extracted at different time-points after addition of LPS.

Determination of miR-23a-5p and inflammatory cytokine level

Total RNA was isolated using a Trizol reagent (Invitrogen, USA). The expression levels of miR-23a-5p and inflammatory cytokine, including TNF-α and IL-1β, were determined by using SYBR-based miRNA quantitative real-time polymerase chain reactions (qRT-PCRs) according to the manufacturer's instructions. The qRT-PCRs were performed by using the ABI PRISM® 7500 Real-Time PCR System. Relative expression of miRNAs was measured on total RNA extracts from the serum using an SYBR Green-based real-time PCR. The sequences for miR-23a-5p, TNF-α and IL-1β, were obtained from the miRBase (http://www.mirbase.org/) and GenBank (http://www.ncbi.nlm.nih.gov/). The primers used in this study were listed in Table 1. The qRT-PCRs were performed by using the ABI PRISM® 7500 Real-Time PCR System. Briefly, 2 µg of miRNA was reversely transcribed using the One Step PrimeScript® miRNA cDNA Synthesis Kit (Takara Biotechnology Co.,

Table 1. Primers used in this study.

Gene names	Accession no.	Primers (5' to 3')
Rat miR-23a-5p	MIMAT0000880	GGGGTTCCTGGGGATGGGATTT
Rat TNF-α	NM_012675.3	Forward: CCAACAAGGAGGAGAAGTTCC Reverse: CTCTGCTTGGTGGTTTGCTAC
Rat IL-1β	NM_031512.2	Forward: GGAACCCGTGTCTTCCTAAAG Reverse: CTGACTTGGCAGAGGACAAAG
Rat GAPDH	NM_017008.4	Forward: TGATTCTACCCACGGCAAGTT Reverse: TGATGGGTTTCCCATTGATGA

Ltd., Japan, D350A). The relative realtime quantitative PCR was performed with SYBR® PremixEx Taq™ (Takara Biotechnology Co., Ltd., Japan, DRR081A) according to the manufacturer's protocol. U6 small nuclear RNA (GeneCopoeia, USA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were selected as internal control genes for the miRNAs and mRNA relative expression assays, respectively. The $^{\Delta\Delta}$Ct method was used to determine the expression level of miR-23a-5p and inflammatory cytokine.

Statistical analysis

The data were obtained from one independent experiment carried out at least triplicate. Interactive and main effects were analyzed by one-way ANOVA using SPSS 18.0 software (SPSS, CA, USA). When justified by one-way ANOVA, differences between individual group means were analyzed by Fisher's PLSD test. Differences were considered statistically significant as $P<0.05$.

Results

General conditions of rats and lung W/D weight ratio

The general conditions of rats in normal control group were good. The rats breathe naturally, move freely, eat regularly, and the response to the stimulation is normal. In LPS group, the rats became agitated restlessly and shortness of breath after injection of LPS for 30 min. After injection of LPS for 3 h, the respiratory symptoms were getting worse. Furthermore, the other manifestations also occurred, including cyanosis of the nose and lip, decrease of muscular tension, weaken response to the stimulation, and diarrhea. Those features meet the criteria of endotoxemia.

The lung W/D weight ratio was used as an important index of water accumulation and the severity of pulmonary edema. From Figure 1 we can see that the lung W/D ratio was highly increased in the rats received LPS (Group 3 h, 6 h, 12 h) compared to the normal control group (Group N) ($P<0.05$), which indicated that the models of acute lung injury in sepsis were induced by administration of LPS.

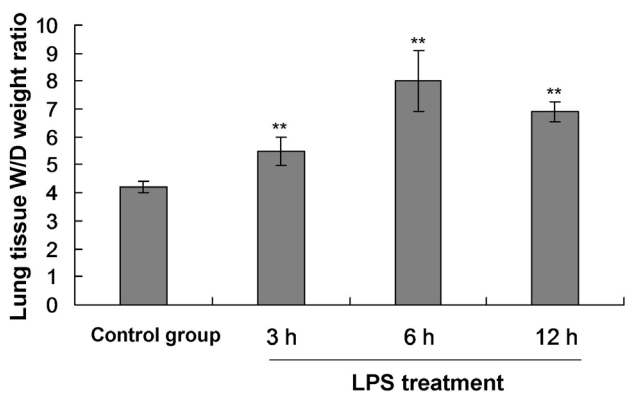

Figure 1. Lung tissue W/D weight ratio (n = 6) between the normal control group and the ARDS model groups (*P<0.05 represents the W/D weight ratio in LPS treated group compared to control group).

Histological findings of Lung injury

The results obtained from light microscopy in the normal control group (group N) showed complete structural integrity, clear alveolar space, and normal alveolar septum. In contrast, extensive pulmonary destruction, including massive inflammatory cell infiltration, pulmonary hemorrhage, obvious thickening of alveolar septum, and consolidation of the lung tissue, was observed in the model groups (group 3 h, 6 h and 12 h) (Figure 2).

miR-23a-5p expresses concurrently with inflammation factors in serum LPS-induced rats

In the models of ARDS, the expression level of miR-23a-5p in the serum was evaluated by qRT-PCR at different time points after LPS induction. The level of miR-23a-5p in serum increased almost 7 fold at 3 h after LPS induction, as shown in Figure 3A. The expression of miR-23a-5p started to decrease at 6 h, and about after 12 h of LPS induction, serum miR-23a-5p dropped to the basal level. Meanwhile, the levels of miR-23a-5p

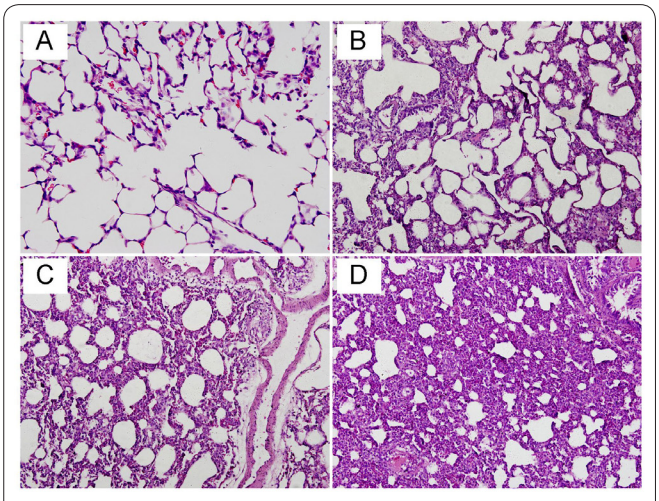

Figure 2. Histological findings in lung tissues stained with hematoxylin and eosin. Images of representative sections from rats lung tissues of control group (A), LPS treatment for 3 h (B), LPS treatment for 6 h (C), LPS treatment for 12 h (D).

in lung tissue were also increased after LPS induction (Figure 3B).

Alongside miR-23a-5p, the levels of the inflammatory cytokines, including TNF-α and IL-1β increased at an early time of 3 h after LPS induction (Figure 4A to 4D). However, except for TNF-α in the serum, the concentrations of the other factors started to decline from 6 h of LPS induction. These data suggest that the miR-23a-5p experienced a transient increase in the serum because of the transient inflammatory stimuli.

Levels of miR-23a-5p and inflammation factors in LPS-induced NR8383 cell

In a separate trial, NR8383 cells were collected after LPS injection. In normal control group, part of the NR8383 cells grew with adherence and the others were in the state of suspension, and grew fast. But after LPS stimulation, the adherent cells gradually decreased, and the suspended cells increased. Meanwhile, the cell growth rate was falling, and the number of live cells decreased (Figure 5A to 5E).

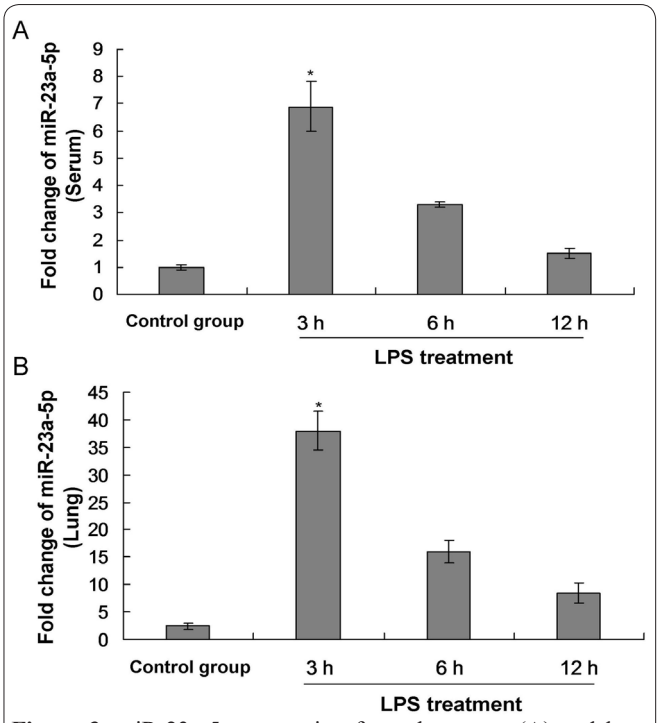

Figure 3. miR-23a-5p expression from the serum (A) and lung tissues (B) of SD rats upon LPS stimulation ($^{*}P<0.05$ represents the fold change of miR-23a-5p in LPS treated group compared to control group).

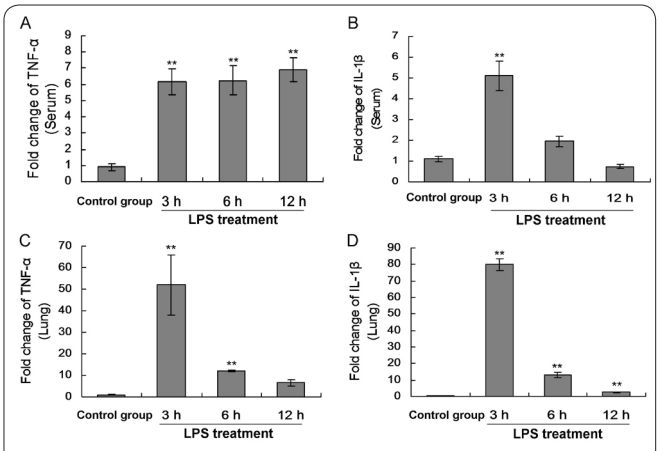

Figure 4. TNF-α and IL-1β mRNA expression in the serum (A and B) and lung tissue (C and D) of SD rat with LPS ($^{**}P<0.01$ represents TNF-α or IL-1β mRNA expression in LPS treated group compared to control group).

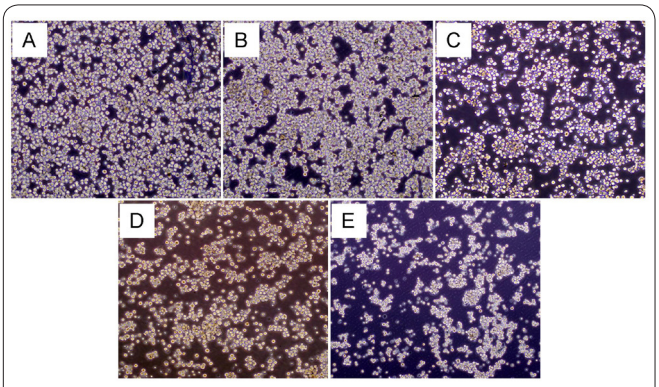

Figure 5. The growth condition and morphological changes of the NR8383 cells after LPS stimulation for control group (A), 3 h (B), 6 h (C), 12 h (D) and 24 h (E).

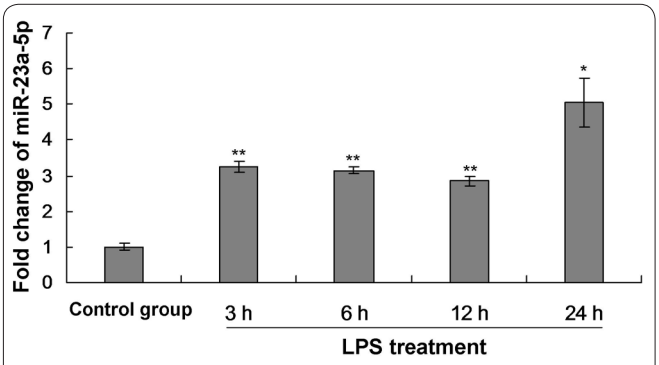

Figure 6. miR-23a-5p expression in the NR8383 cells with LPS ($^{*}P<0.05$ and $^{**}P<0.01$ represent the miR-23a-5p expression in LPS treated group compared to control group).

miR-23a-5p expression significantly increased at 3 h, 6 h 12 h and 24 h of LPS induction, and the first peak was almost 3.5 fold at 3 h after LPS induction (Figure 6). Concurrently, a similar expression pattern of TNF-α and IL-1β was detected with increased inflammatory response in cell (Figure 7A, B). These results further prove that a positive correlation exists between miR-23a-5p and inflammatory factors in ARDS.

Discussion

ARDS is characterized by severe lung inflammation and profound hypoxemia, which also frequently results in multiple organ failure (31), and which is also the major cause of morbidity and mortality. Despite the significant progress has been made in the last decade in the search for biomarkers in clinical ARDS, few biomarkers can be used to predict the progression for diseases, evaluation of response to treatment, risk stratification or prognosis (32). The molecular and immunological mechanisms of ARDS remain incompletely understood. Some components in the circulation such as tissue factor (TF) (33), FITC-Dextran (34), High Mobility Box-1 protein (HMGB1) (35) are recognized as useful biomarkers for the diagnosis and prognosis of ARDS. However, more novel biomarkers with high specificity and sensitivity are highly needed. MicroRNAs are the new class of non-coding RNAs, which participates in a variety of essential disease processes, such as tumorigenesis, natural immunity, self-stabilization, *and ect.* With

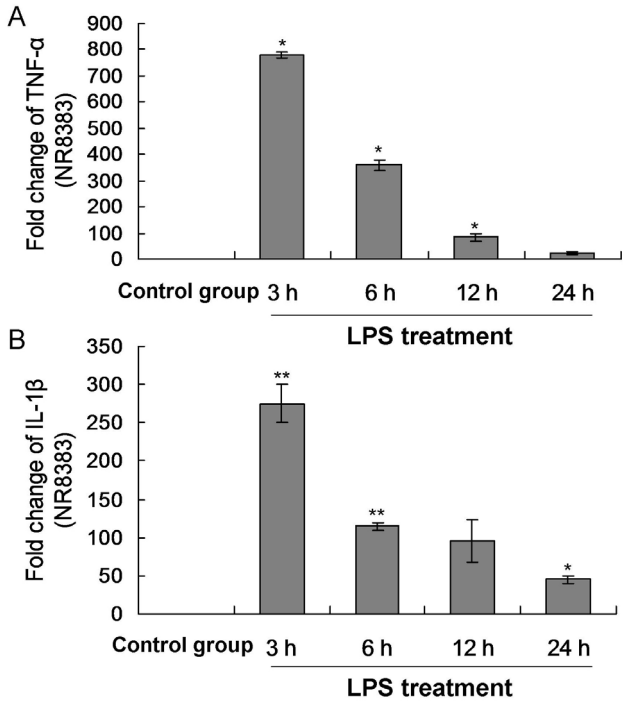

Figure 7. TNF-α and IL-1β mRNA expression in the NR8383 cells with LPS (*P<0.05 and * P<0.01 represent the fold change of TNF-α or IL-1β mRNA expression in LPS treated group compared to control group).

deeper understanding the regulating effects of miRNAs on genetic expression and miRNA differential expression, the potential value of miRNAs in early diagnosis of diseases, therapy and prognosis evaluation were gradually realized. miRNAs were found to be presented in circulation in a remarkably stable form, which can be utilized as good biomarkers. miR-23a-5p is considered to be the optimized candidate for the diagnosis of traumatic injury (22), schizophrenia and schizoaffective disorder (23), and orofacial inflammatory pain (24). However, the expression and significance of miR-23a-5p in ARDS are unclear. Considering some specific miRNAs are stored in form of serum, the advantages of determining miR-23a-5p in serum can be used as biomarkers for ARDS. To our best knowledge, this is the first report providing the direct evidence that serum miR-23a-5p is elevated in ARDS. Our study indicated that miR-23a-5p could be potential biomarkers of this disease.

To better understanding the relationship between miR-23a-5p and sepsis-induced acute respiratory distress syndrome, we determined the expression levels of miR-23a-5p and inflammatory cytokines in serum and lung tissue of ARDS models and NR8383 cells induced by LPS. We found that miR-23a-5p was obviously increased in the serum of ARDS models (LPS group 3 h) and NR8383 cells induced by LPS (LPS group 3 h, 6 h, 12 h, 2 4h) comparing to the normal control group, indicating that miR-23a-5p might be released from injured tissues to the circulation. The general conditions and the lung W/D weight ratio of the rat model, and the histological findings of lung injury were consistent with ARDS. Meanwhile, the inflammatory cytokines, including TNF-α and IL-1β, concurrently increased. Furthermore, the microRNA-23a-5p levels achieved to the peak level 3h after LPS administration. Our study also illustrated the pathological characteristics at 6h after LPS administration. Our study is consistent with the other studies use LPS for ARDS model (36). Therefore, the increase of miR-23a-5p in accordance with the inflammatory response indicates that miR-23a-5p can be a homeostatic response to inflammatory stimuli, which may be useful in the early diagnosis of ARDS.

Furthermore, the extra-cellular signals always cause the changes in the gene expression and metabolism in the cells through the signaling transduction (37). Villar *et al.* (38) discovered that the WNT/β-catenin signaling pathway plays an important role in the ARDS injury. They also proved that some of the signaling pathways, including MAPK pathay, ErbB pathway, DIANA-mirPath pathway and WNT signaling pathway, were controlled by the alteration of the microRNAs in ARDS. Therefore, we speculate that the alterations of microRNA-23a-5p in our study may be also associated with the MAPK, WNT signaling pathway in the pathogenic processes of ARDS. In the future study, we would explore the signaling pathways associating with microRNA-23a-5p and involving in the ARDS.

In this study, we also found the interesting results that the changes of miR-23a-5p were not consistent with the pathology of ARDS processes. Therefore, we think that the miR-23a-5p may act as the potential biomarker for ARDS in early stage, but not related with the progress of disease. In the following study, we would investigate the mechanism of the miR-23a-5p changes and its relationship with the ARDS progress or severity.

Though we have obtained some of the valuable results, there are also a few limitations. In this study, we used the intraperitoneal LPS injection, which resulted in systemic inflammation and high levels of cytokines. However, the high levels of miR-23a-5p were also reported in a few inflammatory diseases, and it's hard to investigate relationship between miR-23a-5p level and ARDS specifically. In the later study, we would establish the ARDS model by intratracheal injection.

In conclusion, we analyzed the expression correlation between miR-23a-5p and inflammatory factors, and found that miR-23a-5p illustrated a highly positive relationship with TNF-α and IL-1β *in vivo* and *in vitro*. These results indicated that miR-23a-5p may serve as a potential novel biomarker for the diagnosis of ARDS in early stage. As serum miR-23a-5p providing new non-invasive windows to the damaged lung tissues, the possibility of using serum miRNA-based assays for early diagnosis of ARDS is opened.

Acknowledgments

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References

1. Goffi, A. and Ferguson, N.D., High-frequency oscillatory ventilation for early acute respiratory distress syndrome in adults. *Curr. Opin. Crit. Care.* 2014, **20:** 77-85. doi: 10.1097/MCC.0000000000000060.

2. Fredenburgh, L.E., Baron, R.M., Crvajal, I.M., Mouded, M., Macias, A.A., Ith, B and Perrella, M.A., Absence of heme oxygenase-1 expression in the lung parenchyma exacerbates endotoxin-

induced acute lung injury and decreases surfactant protein-B levels. *Cell. Mol. Biol. (Noisy-le-grand).* 2005, **51:** 513-520.

3. Binnie, A., Tsang, J.L. and dos Santos, C,C., Biomarkers in acute respiratory distress syndrome. *Curr. Opin. Crit. Care.* 2014, **20:** 47-55. doi: 10.1097/MCC.000000000 0000048.

4. Terpstra, M.L., Aman, J., van Nieuw Amerongen, G.P. and Groeneveld, A.B., Plasma biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis. *Crit. Care. Med.* 2014, **42:** 691-700. doi: 10.1097/01.ccm.0000435669.60811.24.

5. Janz, D.R. and Ware, L.B., Biomarkers of ALI/ARDS: pathogenesis, discovery, and relevance to clinical trials. *Semin. Respir. Crit. Care Med.* 2013, **34:** 537-548. doi: 10.1055/s-0033-1351124.

6. Cross, L.J. and Matthay, M.A., Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury. *Crit. Care Clin.* 2011, **27:** 355-377. doi: 10.1016/j.ccc.2010.12.005.

7. Gaggar, A. and Olman, M.A., Biologic markers of mortality in acute lung injury. *Clin. Chim. Acta.* 2006, **372:** 24-32.

8. Benfey, P.N., Molecular biology: microRNA is here to stay. *Nature.* 2003, **425:** 244-245.

9. Kozomara, A. and Griffiths-Jones, S.. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic. Acids Res.* 2011, **39:** D152-157. doi: 10.1093/nar/gkq1027.

10. He, L., Thomson, J.M., Hemann, M.T., Hernando-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S.W., Hannon, G.J. and Hammond, S.M., A microRNA polycistron as a potential human oncogene. *Nature.* 2005, **435:** 828-833.

11. Hatfield, S.D., Shcherbata, H.R., Fischer, K.A., Nakahara, K., Carthew, R.W. and Ruohola-Baker, H., Stem cell division is regulated by the microRNA pathway. *Nature.* 2005, **435:** 974-978.

12. Triboulet, R., Mari, B., Lin, Y.L., Chable-Bessia, C., Bennasser, Y., Lebrigand, K., Cardinaud, B., Maurin, T., Barbry, P., Baillat, V., Reynes, J., Corbeau, P., Jeang, K.T. and Benkirane, M., Suppression of microRNA-silencing pathway by HIV-1 during virus replication. *Science.* 2007, **315:** 1579-1582.

13. Kim, J., Inoue, K., Ishii, J., Vanti, W,B,, Voronov, S,V., Murchison, E., Hannon, G. and beliovich, A., A MicroRNA feedback circuit in midbrain dopamine neurons. *Science.* 2007, **317:** 1220-1224.

14. Yang, Y., Cheng, H., Qiu, Y., Dupee, D., Noonan, M., Lin, Y.D., Fisch, S., Unno, K., Sereti, K.I. and Liao, R., MicroRNA-34a Plays a Key Role in Cardiac Repair and Regeneration Following Myocardial Infarction. *Circ, Res.* 2015, **117:** 450-459. doi: 10.1161/CIRCRESAHA.117.305962.

15. Takahashi, R.U., Miyazaki, H., Takeshita, F., Yamamoto, Y., Minoura, K., Ono, M., odaira, M., Tamura, K., Mori, M., Ochiya, T., Loss of microRNA-27b contributes to breast cancer stem cell generation by activating ENPP1. *Nat. Commun.* 2015, **6:** 7318. doi: 10.1038/ncomms8318.

16. Peng, H., Liu, Y., Tian, J., Ma, J., Tang, X., Yang, J., Rui, K., Zhang, Y., Mao, C., Lu, L., Xu, L., Xu. H. and Wang, S., Decreased expression of microRNA-125a-3p upregulates interleukin-23 receptor in patients with Hashimoto's thyroiditis. *Immunol. Res.* 2015, **62:** 129-136. doi: 10.1007/s12026-015-8643-3.

17. Matsumura, T., Sugimachi, K., Iinuma, H., Takahashi, Y., Kurashige, J., Sawada, G., Ueda, M., Uchi, R., Ueo, H., Takano, Y., Shinden, Y., Eguchi, H., Yamamoto, H., Doki, Y., Mori, M., Ochiva, T. and Mimori, K., Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. *Br. J. Cancer.* 2015, **113:** 275-281. doi: 10.1038/bjc.2015.201.

18. Lawrie, C.H., Gal, S., Dunlop, H.M., Pushkaran, B., Liggins, A.P., Pulford, K., Banham, A.H., Pezzella, F., Boultwood, J., Wainscoat, J.S., Hatton, C.S. and Harris, A.L., Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br. J. Haematol. 2008, 141: 672-675.

19. Xiao J, Jing ZC, Ellinor PT, Liang D, Zhang H, Liu Y, Chen X, Pan L, Lyon R, Liu Y, Peng LY, Liang X, Sun Y, Popescu LM, Condorelli G, Chen YH. MicroRNA-134 as a potential plasma biomarker for the diagnosis of acute pulmonary embolism. *J. Transl. Med.* 2011; **9:** 159. doi: 10.1111/j.1365-2141.2008.07077.x.

20. Yu, X. and Li, Z., The role of MicroRNAs expression in laryngeal cancer. *Oncotarget.* 2015, **6:** 23297-23305.

21. Powrózek, T., Krawczyk, P., Kowalski, D.M., Winiarczyk, K., Olszyna-Serementa, M. and Milanowski, J., Plasma circulating microRNA-944 and microRNA-3662 as potential histologic type-specific early lung cancer biomarkers. *Transl. Res.* 2015, **166:** 315-323. doi: 10.1016/j.trsl.2015.05.009.

22. Yang, J., Liang, Y., Han, H. and Qin, H., Identification of a miRNA signature in neutrophils after traumatic injury. *Acta. Biochim. Biophys. Sin (Shanghai).* 2013; **45:** 938-945. doi: 10.1093/abbs/gmt100.

23. Zhao, D., Lin, M., Chen, J., Pedrosa, E., Hrabovsky, A., Fourcade, H.M., Zheng, D. and Lachman, H.M., MicroRNA Profiling of Neurons Generated Using Induced Pluripotent Stem Cells Derived from Patients with Schizophrenia and Schizoaffective Disorder, and 22q11.2 Del. *PLoS One.* 2015, **10:** e0132387. doi: 10.1371/journal.pone.0132387.

24. Dong, Y., Li, P., Ni, Y., Zhao, J. and Liu, Z., Decreased microRNA-125a-3p contributes to upregulation of p38 MAPK in rat trigeminal ganglions with orofacial inflammatory pain. *PLoS One.* 2014, **9:** e111594. doi: 10.1371/journal.pone.0111594.

25. Zeng, Z., Gong, H., Li, Y., Jie, K., Ding, C., Shao, Q., Liu, F., Zhang, Y., Nie, C., Zhu, W. and Qian, K., Upregulation of miR-146a contributes to the suppression of inflammatory responses in LPS-induced acute lung injury. *Exp. Lung Res.* 2013, **39:** 275-282. doi: 10.3109/01902148.2013.808285.

26. Huang, C., Xiao, X., Chintagari, N.R., Breshears, M., Wang, Y. and Liu, L., MicroRNA and mRNA expression profiling in rat acute respiratory distress syndrome. *BMC. Med. Genomics.* 2014, **7:** 46. doi: 10.1186/1755-8794-7-46.

27. Guo, Z., Gu, Y., Wang, C., Zhang, J., Shan, S., Gu, X., Wang, K., Han, Y. and Ren, T., Enforced expression of miR-125b attenuates LPS-induced acute lung injury. *Immunol. Lett.* 2014, **162:** 18-26. doi: 10.1016/j.imlet.2014.06.008.

28. Katzenstein, A.L., Bloor, C.M. and Leibow, A.A., Diffuse alveolar damage- the role of oxygen, shock, and related factors. A review. *Am. J. Pathol.* 1976, **85:** 209-228.

29. Thille, A.W., Esteban, A. and Fernandez-Segoviano P., Comparison of the Berlin definition for respiratory distress syndrome with autopsy. *Am. J. Respir. Crit. Care. Med.* 2013, **187:** 761-767. doi: 10.1164/rccm.201211-1981OC.

30. Chi, X., Zhang, A., Luo, G., Xia, H., Zhu, G., Hei, Z., Liu, X., Wei, J. and Xia, Z., Knockdown of myeloid differentiation protein-2 reduces acute lung injury following orthotopic autologous liver transplantation in a rat model. *Pulm. Pharmacol. Ther.* 2013, **26:** 380-387. doi: 10.1016/j.pupt.2013.02.001.

31. Wheeler, A.P. and Bernard, G.R., Acute lung injury and the acute respiratory distress syndrome: a clinical review. *Lancet.* 2007, **369:** 1553-1564.

32. Barnett, N. and Ware, L.B., Biomarkers in acute lung injury--marking forward progress. *Crit. Care Clin.* 2011, **27:** 661-683. doi: 10.1016/j.ccc.2011.04.001.

33. Xue, M., Sun, Z., Shao, M., Yin, J., Deng, Z., Zhang, J., Xing, L., Yang, X., Chen, B., Dong, Z., Han, Y., Sun, S., Wang, Y., Yao, C., Chu, X., Tong, C. and Song, Z., Diagnostic and prognostic utility of tissue factor for severe sepsis and sepsis-induced acute lung injury. *J. Transl. Med.* 2015; **13:** 172. doi: 10.1186/s12967-015-0518-9.

34. Chen, H., Wu, S., Lu, R., Zhang, Y.G., Zheng, Y. and Sun, J., Pulmonary permeability assessed by fluorescent-labeled dextran instilled intranasally into mice with LPS-induced acute lung injury.

PLoS One. 2014, **9:** e101925. doi: 10.1371/journal. pone.0101925.
35. Cohen, M.J., Brohi, K., Calfee, C.S., Rahn, P., Chesebro, B.B., Christiaans, S.C., Carles, M., Howard, M. and Pittet, J.F., Early release of high mobility group box nuclear protein 1 after severe trauma in humans: role of injury severity and tissue hypoperfusion. *Crit. Care.* 2009, **13:** R174. doi: 10.1186/cc8152.
36. Oliveira, M.C., Greiffo, F.R., Rigonato-Oliveira, N.C., Custodio, R.W., Silva, V.R., Damaceno-Rodrigues, N.R., Almeida, F.M., Albertini, R., Lopes-Martins, R.A., Oliveira, L.V., de Carvalho Pde, T., Ligeiro de Oliveira, A.P., Leal, E.C. and Vieira, R.P., Low level laser therapy reduces acute lung inflammation in a model of pulmonary and extrapulmonary LPS-induced ARDS. *J. Photochem. Photobiol. B.* 2014, **134:** 57-63. doi: 10.1016/j.jphotobiol.2014.03.021.
37. Huang, C., Xiao, X., Chintagari, N.R., Breshears, M., Wang, Y., Liu, L., MicroRNA and mRNA expression profiling in rat acute respiratory distress syndrome. *BMC. Med. Genomics.* 2014; **7:** 46. doi: 10.1186/1755-8794-7-46.
38. Villar, J., Cabrera, N.E., Casula, M., Valladares, F., Flores, C., Lopez-Aguilar, J., Blanch, L., Zhang, H., Kacmarek, R.M., Slutsky, A.S., WNT/beta-catenin signaling is modulated by mechanical ventilation in an experimental model of acute lung injury. *Intensive. Care. Med.* 2011; **7:** 1201-1209. doi: 10.1007/s00134-011-2234-0.