MiR-199a-3p Attenuates Sepsis-Induced Acute Lung Injury by Targeting NLRP1 and Suppressing Caspase-1/GSDMD-Mediated Pyroptosis in a CLP Rat Model

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Abstract: Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome (ARDS), remain major causes of mortality in critical care. MicroRNAs play essential roles in regulating inflammation and programmed cell death. In this study, miR-199a-3p was identified as one of the most significantly downregulated miRNAs in ALI. Bioinformatic and luciferase assays confirmed NLRP1 as its direct target. Using a cecal ligation and puncture (CLP) rat model, we found that CLP markedly decreased miR-199a-3p expression while upregulating NLRP1, cleaved Caspase-1, and GSDMD-N, leading to severe lung injury. Lentiviral overexpression of miR-199a-3p significantly reduced pulmonary inflammation, cytokine release, and histopathological damage by suppressing the NLRP1/Caspase-1/GSDMD pathway. These results demonstrate that miR-199a-3p protects against sepsis-induced ALI by inhibiting pyroptosis through direct targeting of NLRP1, suggesting its potential as a therapeutic target for ALI/ARDS.

Keywords: miR-199a-3p; NLRP1; Pyroptosis; Acute Lung Injury; Sepsis; Caspase-1; GSDMD; Cecal Ligation and Puncture (CLP)

1. Introduction

Acute lung injury (ALI) and its severe manifestation, acute respiratory distress syndrome (ARDS), continue to pose significant challenges in critical care, with mortality remaining as high as 30–40% despite advances in ventilation techniques and supportive treatments [1,2]. The underlying mechanisms of ALI involve excessive inflammation, damage to epithelial and endothelial barriers, and aberrant forms of programmed cell death. Nevertheless, specific molecular-targeted therapies are still lacking, emphasizing the need for deeper mechanistic understanding and novel therapeutic approaches.

MicroRNAs (miRNAs), a class of short non-coding RNAs of approximately 18–25 nucleotides, play pivotal roles in post-transcriptional gene regulation and inflammatory signaling [3,4]. Several miRNAs, such as miR-21, miR-155, miR-146a, and miR-223, have been implicated in sepsis-associated organ dysfunction [5,6]. Among them, miR-199a-3p has attracted increasing attention for its involvement in hypoxia adaptation, apoptosis, and inflammation-related pathways in cardiovascular and pulmonary disorders [7,8]. However, its precise role in ALI/ARDS remains poorly defined.

Our preliminary investigations revealed a pronounced downregulation of miR-199a-3p in lung tissues obtained from ALI patients and CLP-induced rat models, as validated by microarray profiling and RT-qPCR. Computational analyses using TargetScan, miRDB, and StarBase identified nucleotide-binding oligomerization domain-like receptor protein 1 (NLRP1) as a potential direct target of miR-199a-3p, and dual-luciferase reporter assays in A549 and U937 cells confirmed this regulatory interaction. These observations suggest that miR-199a-3p may influence inflammasome-mediated pyroptosis through modulation of NLRP1 expression.

NLRP1, a key component of the Nod-like receptor (NLR) inflammasome family, functions as an important upstream trigger of Caspase-1 activation. Through this pathway, it promotes the conversion of pro-IL-1β and pro-IL-18 into their active, mature forms and induces membrane pore

formation by mediating the cleavage of gasdermin D (GSDMD) [1]. Overactivation of the NLRP1 inflammasome leads to pyroptotic cell death, an inflammatory form of programmed cell death that plays a critical role in epithelial injury and alveolar barrier dysfunction during sepsis-induced acute lung injury (ALI). Persistent stimulation of this pathway can result in excessive cytokine release, aggravating tissue destruction and amplifying systemic inflammation.

While the NLRP3 inflammasome has been widely investigated in the context of ALI and various inflammatory disorders, relatively little is known about the contribution of NLRP1 to this process or how its activation is regulated. Notably, NLRP1 differs from NLRP3 in its activation mechanism—responding more specifically to microbial toxins, metabolic stress, and pathogen-associated molecular patterns—indicating that it may act as a unique sensor of cellular damage in pulmonary tissue. Despite its potential significance, the post-transcriptional mechanisms that fine-tune NLRP1 activity remain largely undefined. Increasing evidence suggests that microRNAs (miRNAs) can serve as essential regulatory molecules in controlling inflammasome-related signaling by modulating gene expression at the mRNA level. However, the specific involvement of miRNAs in targeting NLRP1 during sepsis-related inflammatory injury has not been fully elucidated. Exploring the role of miR-199a-3p in this regulatory network may therefore provide valuable insights into novel molecular targets for preventing pyroptosis-driven lung damage in septic conditions [2,9].

Building on these findings, we proposed that restoration of miR-199a-3p expression could mitigate NLRP1-dependent pyroptosis and thereby alleviate sepsis-induced lung injury [10]. To test this hypothesis, a CLP-induced ALI rat model was established, and a lentiviral vector was used to overexpress miR-199a-3p in vivo. The extent of lung injury, cytokine release, and pyroptosis-associated molecular changes were subsequently evaluated.

Collectively, this work provides the first evidence that miR-199a-3p ameliorates sepsis-associated ALI through direct targeting of NLRP1, uncovering a previously unrecognized regulatory axis in inflammatory lung injury and offering a promising RNA-based therapeutic perspective.

2. Materials and Methods

2.1 Laboratory Animal

Male Sprague–Dawley rats weighing 200–220g were purchased from the Experimental Animal Center of Nanchang Medical College. All experimental protocols were conducted in accordance with the guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals.

2.2 Experimental Groups

Rats were randomly assigned to four groups (n = 8 per group):

1.Sham: laparotomy without CLP + LV-NC
2.CLP: CLP + LV-NC
3.CLP + LV-NC: CLP + empty lentiviral vector
4.CLP + LV-miR-199a-3p: CLP + miR-199a-3p overexpression vector

2.3 Lentiviral Vector Administration

A recombinant lentiviral vector encoding the precursor sequence of miR-199a-3p (LV-miR-199a-3p) or a negative control sequence (LV-NC) was generated by Fosum Biotechnology (China). Each rat was injected with 2×10^8 transducing units (TU) of lentivirus through the tail vein 7 days before the CLP procedure to ensure stable miR-199a-3p overexpression prior to model establishment.

2.4 CLP-Induced ALI Model

The cecal ligation and puncture (CLP) procedure was conducted as previously reported (Ref). Rats were anesthetized with 3% isoflurane, after which the cecum was ligated at approximately one-third of its length from the distal end, punctured twice using an 18-gauge needle, and gently pressed to extrude a small amount of fecal material. In the Sham group, identical surgical steps were performed without ligation or puncture. Lung tissues and serum samples were collected 24 hours after CLP for subsequent analyses.

2.5 Sample Collection

At 24 h post-operation, rats were euthanized; blood samples were collected for ELISA, and lung tissues were divided for histology, RT-qPCR, Western blot, and immunohistochemistry.

3. Results

3.1 miR-199a-3p is Significantly Downregulated in ALI and Restored by Lentiviral Overexpression

RT-qPCR analysis showed that miR-199a-3p expression was markedly reduced in the lung tissues of CLP rats compared with the Sham group (0.22 ± 0.05 vs 1.00 ± 0.09, $p < 0.001$). Administration of the empty lentiviral vector (LV-NC) did not alter this reduction (0.25 ± 0.06, $p > 0.05$ vs CLP). In contrast, delivery of LV-miR-199a-3p significantly restored miR-199a-3p levels (0.79 ± 0.08, $p < 0.001$ vs CLP), indicating successful *in vivo* overexpression (Figure 1.).

These findings confirmed that CLP-induced ALI suppresses endogenous miR-199a-3p, while lentiviral pre-treatment effectively reverses this reduction.

3.2 miR-199a-3p Directly Targets NLRP1 in Rat Lung Tissue

Western blot and RT-qPCR results demonstrated a reciprocal relationship between miR-199a-3p and NLRP1 expression. CLP markedly upregulated NLRP1 mRNA (4.3-fold, $p < 0.001$) and protein levels ($p < 0.001$ vs Sham). LV-miR-199a-3p administration significantly reduced NLRP1 mRNA (−61%) and protein expression (−64%) compared with the CLP group ($p < 0.01$) (Figure 2.).

These data support the post-transcriptional inhibitory effect of miR-199a-3p on NLRP1 observed in vitro.

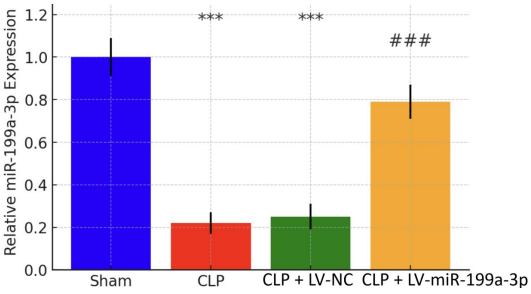

Figure 1. MiR-199a-3p Relative Expression

3.3 Overexpression of miR-199a-3p Ameliorates CLP-Induced Lung Injury

Histopathological examination revealed normal alveolar structure in Sham rats, whereas CLP rats displayed alveolar wall thickening, massive neutrophil infiltration, and interstitial edema. Lung injury scores were significantly higher in the CLP group (9.1 ± 0.8 vs 1.2 ± 0.3, $p < 0.001$). LV-miR-199a-3p treatment reduced pathological injury (score 4.0 ± 0.7, $p < 0.001$ vs CLP), while LV-NC exerted no protective effect (8.6 ± 0.6, $p > 0.05$) (Figure 3.).

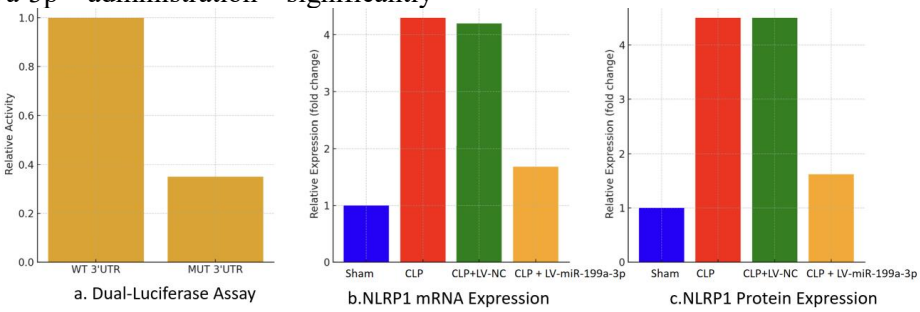

Figure 2. Dual-Luciferase Assay and NLRP1 Expression Change

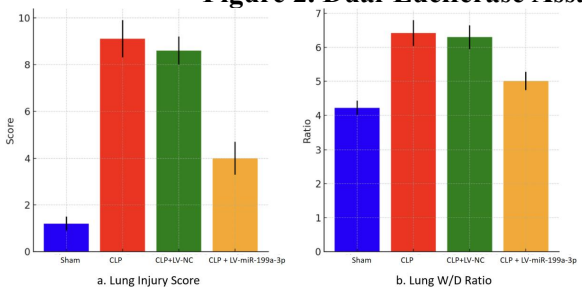

Figure 3. Lung Injury Score and W/D Ratio

Similarly, the lung wet/dry ratio increased after CLP (6.42 ± 0.38 vs 4.22 ± 0.21, $p < 0.001$), but was significantly decreased following miR-199a-3p overexpression (5.01 ± 0.27, $p < 0.01$).

3.4 MiR-199a-3p Inhibits Activation of the NLRP1/Caspase-1/GSDMD Pyroptosis Pathway

Western blot analysis showed strong induction of cleaved Caspase-1 (p20) and GSDMD-N in CLP lungs, indicating activation of pyroptosis. Compared with Sham, CLP caused a 5.1-fold increase in cleaved Caspase-1 and 4.7-fold increase in GSDMD-N ($p < 0.001$). LV-miR-199a-3p markedly suppressed these increases (−58% and −54%, respectively; $p < 0.01$) (Figure 4.).

No such inhibition was observed in the LV-NC group.

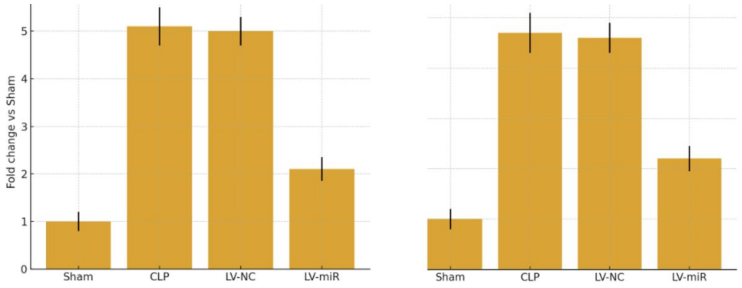

Figure 4. Densitometry Bar Chart

3.5 Immunohistochemistry Confirms Reduced NLRP1 and Pyroptosis Markers after miR-199a-3p Treatment

IHC staining showed low basal NLRP1 expression in Sham rats, whereas dense brown staining was observed in CLP lungs, particularly in alveolar epithelium. IOD analysis showed a 4.6-fold increase in NLRP1 staining ($p < 0.001$) (Figure 5.).

LV-miR-199a-3p significantly decreased NLRP1 staining intensity by 63% ($p < 0.01$). Similar reductions were observed for GSDMD.

3.6 Serum Inflammatory Cytokines are Attenuated by miR-199a-3p Overexpression

ELISA showed that CLP markedly elevated serum IL-1β (5.8×), IL-18 (4.9×), and TNF-α (3.7×) compared with Sham ($p < 0.001$) (Figure 6.).

LV-miR-199a-3p significantly reduced all three cytokines by 45–60% ($p < 0.01$), consistent with suppression of inflammasome activation.

LV-NC showed no change.

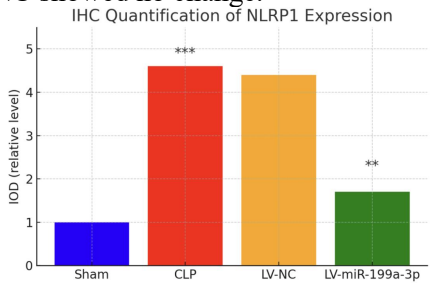

Figure 5. IOD Histogram

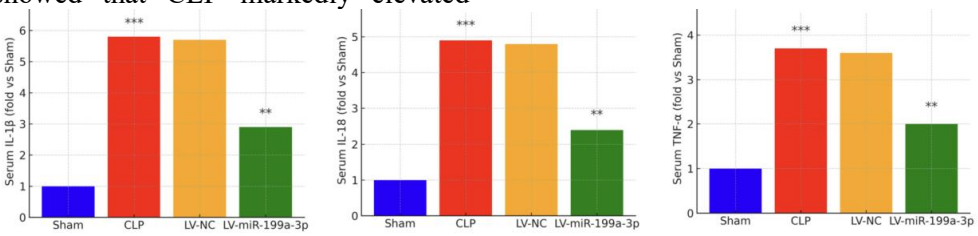

Figure 6. ELISA Bar Graphs for 3 Cytokines

3.7 Proposed Mechanism Model

The data support a mechanistic model in which CLP suppresses miR-199a-3p, leading to upregulation of NLRP1, activation of Caspase-1, cleavage of GSDMD, and pyroptotic lung injury. Restoration of miR-199a-3p blocks this pathway and confers lung protection.

4. Discussion

In the present study, we demonstrated that miR-199a-3p acts as a critical negative regulator of NLRP1-mediated pyroptosis in sepsis-induced acute lung injury. Using a CLP rat model and in vivo lentiviral delivery, we confirmed that miR-199a-3p is markedly downregulated in ALI, resulting in upregulation of NLRP1, activation of Caspase-1, cleavage of GSDMD, and subsequent lung tissue damage. Restoration of miR-199a-3p expression significantly attenuated lung inflammation, reduced pyroptosis-related protein activation, improved histological injury, and decreased systemic cytokine release [11]. These findings establish the miR-199a-3p/NLRP1 axis as a mechanistic link between sepsis and pyroptotic lung injury, suggesting its potential as a therapeutic target for ALI/ARDS.

4.1 miR-199a-3p as a Regulator of Inflammatory Injury in ALI

miR-199a-3p has been widely recognized as a multifunctional regulator involved in diverse biological and pathological processes, including inflammation, apoptosis, oxidative stress, and fibrosis. Numerous experimental and clinical studies have demonstrated that this microRNA

plays a critical protective role in various organ systems. For instance, in hepatic injury, miR-199a-3p attenuates inflammatory cytokine release and prevents hepatocellular apoptosis by interfering with NF-κB activation and related inflammatory transcriptional programs. Similarly, in myocardial ischemia and reperfusion injury, miR-199a-3p has been shown to reduce oxidative stress and fibrosis through negative regulation of the TGF-β/Smad and MAPK signaling pathways, thereby preserving cardiomyocyte survival and cardiac function. In the context of malignancy, this miRNA can function as a tumor suppressor, inhibiting proliferation, migration, and epithelial–mesenchymal transition by targeting oncogenic signaling nodes such as HIF-1α, mTOR, and c-Met. Collectively, these findings indicate that miR-199a-3p exerts broad cytoprotective effects by fine-tuning multiple proinflammatory and profibrotic cascades.

Despite this extensive evidence in other disease models, the specific role of miR-199a-3p in acute lung injury (ALI) has remained poorly characterized. The lung is uniquely vulnerable to systemic inflammatory insults, particularly in sepsis, where dysregulated immune activation and cytokine storm lead to alveolar-capillary barrier disruption. In our present study, we detected a significant downregulation of miR-199a-3p expression both in previously published clinical samples from ALI patients and in experimental rats subjected to cecal ligation and puncture (CLP), a well-established sepsis model. This consistent suppression across species and models suggests that loss of miR-199a-3p may be a conserved pathological response to overwhelming inflammation and septic challenge. Such reduction may relieve inhibition on downstream proinflammatory mediators, amplifying inflammasome activation, cytokine release, and tissue injury. Therefore, our findings highlight miR-199a-3p as a potential upstream modulator of inflammatory homeostasis in sepsis-induced ALI and warrant further investigation into its therapeutic and diagnostic value.

This reduction likely contributes to the amplification of inflammatory responses and programmed cell death in lung tissue. Extending previous in vitro studies, our findings provide the first in vivo confirmation that miR-199a-3p directly modulates inflammasome-driven pyroptosis in the lung. Mechanistically, diminished miR-199a-3p expression lifts inhibitory control over NLRP1, promoting Caspase-1 activation, GSDMD cleavage, and subsequent release of pro-inflammatory mediators. Reintroduction of miR-199a-3p effectively reversed these changes, alleviating tissue injury and dampening cytokine release [12]. Collectively, these results indicate that miR-199a-3p functions as a critical post-transcriptional regulator of inflammasome activity and pyroptotic signaling, offering new insights into its potential as a therapeutic modulator in sepsis-induced ALI.

4.2 NLRP1 Inflammasome: a Neglected Mediator of Pulmonary Pyroptosis

Most ALI/ARDS pyroptosis studies to date have focused on the NLRP3 inflammasome, while NLRP1 has received comparatively little attention, despite being the first inflammasome protein identified. Here, we demonstrate that NLRP1 is strongly induced in septic lung tissue and functions upstream of Caspase-1 and GSDMD activation. Our findings are consistent with emerging reports that NLRP1 contributes to epithelial pyroptosis, ventilator-induced lung injury, and COVID-19-associated hyperinflammation. By identifying a miRNA that directly suppresses NLRP1, this study adds a new regulatory layer to inflammasome biology and supports the concept that NLRP1, not only NLRP3, participates in lung injury.

4.3 Mechanistic Significance of the miR-199a-3p/NLRP1/Caspase-1/GSDMD Axis

Pyroptosis, an inflammatory form of programmed cell death, plays a key role in the pathogenesis of sepsis-induced lung injury. This process involves inflammasome activation, Caspase-1 cleavage, and GSDMD-mediated pore formation, ultimately leading to cell rupture and the release of pro-inflammatory cytokines such as IL-1β and IL-18. In this study, rats exposed to cecal ligation and puncture (CLP) exhibited robust activation of cleaved Caspase-1 (p20) and GSDMD-N, confirming pyroptotic signaling in lung tissues. Overexpression of miR-199a-3p via lentiviral delivery markedly suppressed these changes, reducing both the protein levels of pyroptosis markers and cytokine secretion, which corresponded with attenuated tissue damage [13]. Mechanistically, the downregulation of miR-199a-3p under septic

conditions removes its inhibitory control over NLRP1, facilitating inflammasome assembly, Caspase-1 activation, and GSDMD-driven membrane perforation. Restoration of miR-199a-3p expression interrupts this sequence, dampening excessive inflammation and protecting alveolar structures. Overall, these findings demonstrate that the miR-199a-3p/NLRP1/Caspase-1/GSDMD signaling cascade is a central mediator of pyroptotic injury, with miR-199a-3p acting as a crucial upstream regulator and potential therapeutic target to mitigate inflammasome-induced pulmonary damage.

4.4 Translational Implications

Despite advances in critical care, the mortality rate of acute respiratory distress syndrome (ARDS) remains above 30%, and there is still no effective molecular-targeted therapy capable of addressing its underlying mechanisms. MicroRNAs have recently attracted attention as potential therapeutic agents because of their high specificity, stability, and capacity to regulate multiple targets within interconnected signaling pathways. In this study, the protective effects of miR-199a-3p manifested as reduced histological damage, lower inflammatory cytokine levels, and suppression of inflammasome activity, suggest its potential as both a circulating biomarker and a candidate for RNA-based therapeutic development in sepsis-induced ALI [14].

From a translational perspective, restoring miR-199a-3p expression could provide a novel approach for modulating excessive inflammation and pyroptotic cell death. Therapeutically, miR-199a-3p mimics might be incorporated into nanoparticle-based or viral delivery systems to achieve targeted re-expression in lung tissue, while serum miR-199a-3p levels may serve as a useful indicator of disease progression or treatment efficacy. Moreover, since pyroptosis is also implicated in other sepsis-related organ injuries, such as acute kidney injury, myocardial dysfunction, and central nervous system damage, the regulatory role of miR-199a-3p may extend beyond the pulmonary system. Therefore, therapeutic modulation of miR-199a-3p could represent a unifying strategy for limiting systemic inflammation and multiple organ injury in sepsis, highlighting its potential value in precision medicine for critical illness.

4.5 Limitations and Future Perspectives

This study has several limitations. First, only miR-199a-3p overexpression was evaluated; loss-of-function rescue experiments (e.g., antagomir or NLRP1 knockdown) were not performed. Second, although CLP is the gold standard animal model for sepsis, validation in human samples or organoids will be required to confirm clinical relevance. Third, this study focused on NLRP1 but did not assess potential crosstalk with other inflammasomes such as NLRP3 or AIM2. Future studies should include dual-target models, time-course pyroptosis imaging, and combined miR-199a-3p + NLRP1-siRNA therapy to confirm causality. Finally, pharmacologic delivery of miR-199a-3p mimics (e.g., nanoparticle-based) should be explored as a clinically feasible treatment strategy.

4.6 Summary of Scientific Contribution

Overall, this study provides the first in vivo evidence that restoring miR-199a-3p expression protects against septic ALI by directly targeting NLRP1 and suppressing pyroptosis. These findings position the miR-199a-3p/NLRP1 axis as a novel regulatory pathway in sepsis-associated lung injury and support further investigation of miRNA-based therapeutic strategies in critical care medicine.

5. Conclusion

This study demonstrates that miR-199a-3p is significantly downregulated in sepsis-induced acute lung injury, leading to activation of the NLRP1/Caspase-1/GSDMD pyroptosis pathway. Lentiviral overexpression of miR-199a-3p restores homeostatic regulation, suppresses NLRP1 expression, reduces inflammatory cytokine release, and alleviates lung tissue damage. These findings identify miR-199a-3p as a key upstream regulator of inflammasome-mediated lung injury and suggest that targeting the miR-199a-3p/NLRP1 axis may represent a promising therapeutic strategy for ALI/ARDS.

Acknowledgments

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