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Overexpressed miR-539 exacerbates *Pseudomonas aeruginosa* puenmonia by promoting inflammatory responses

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Dear Editor,

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium that causes a series of life-threatening acute and/or chronic infections in humans, often in persons with immunodeficiency. *P. aeruginosa* has been listed as one of the priority bacteria that requires extensive research and urgent development of new antibiotic treatments by the World Health Organization (WHO) in 2017.[1] In recent years, numerous studies have shown that miRNAs play an important role in infection and inflammatory responses.[2,3] Our earlier researches suggest that miRNAs are a key regulator of the inflammatory responses and are involved in the clearance of *P. aeruginosa in vitro* and *in vivo*.[4–6] In this study, we demonstrated that miR-539 expression was significantly increased after *P. aeruginosa* infection *in vitro* and *in vivo* (Supplymentary Materials and Methods). Critically, overexpression of miR-539 promoted the expression of inflammatory cytokines, causing severe lung injury and high death rates in mice with *P. aeruginosa* pneumonia.

Here, we discovered that compared to uninfected controls, miR-539 was upregulated in MH-S cells and in mice after *P. aeruginosa* infection (Fig. 1A). To determine the potential role of miR-539 in bacterial infection, we further studied the effect of miR-539 on bacterium-induced inflammatory cytokine gene expression in MH-S cells. Chemically synthesized mimics (539-m) increased bacterium-induced TNF-α and IL-1β mRNA expression by more than 50% compared to miRNA mimic negative controls (NC-m), while miR-539 inhibitors (539-i) inhibited these cytokines' expression by more than 60% compared to miRNA inhibitors negative controls (NC-i), as assessed by RT-qPCR or Western blotting, respectively (Fig. 1B, C). Interestingly, miR-539 expression was decreased in cells overexpressing miR-539 after PA14 infection, which may be caused by intracellular depletion and degradation of this miRNA over time (Fig. 1B). Built on the data of MH-S cells, we reasoned that miR-539 may also function similarly *in vivo* and tested this hypothesis in a mouse model of acute infection. Six-week-old female C57BL/6J mice were injected i.v. with 539-m or NC-m and then challenged with PA14 (CFU 5×10^6). Next, we isolated macrophages within the lung tissues by cell adherence method. Flow cytometry analysis showed that approximately 80% adherent cells were macrophages. Furthermore, miR-539 mimics increased the expression of TNF-α mRNA and IL-1β mRNA in AMs compared to those in control mimics-transfected cells (Fig. 1D). In addition, systemic delivery of miR-539 mimics increased miR-539 expression in mouse lung tissues and PA14 infection reduced the levels of miR-539 (Fig. 1E). This was also similarly shown in cells (Fig. 1B). Importantly, expression of TNF-α and IL-1β mRNA also increased by about 50% in the lungs of miR-539 overexpressing mice (Fig. 1E). To better understand the overall effect of miR-539 on *P. aeruginosa*-infected lungs and animal survivals, we performed lung histopathological analysis and survival studies in mice. As shown in Fig. 1F, PA14 infection induced strong inflammatory responses and leukocyte infiltration in 539-m-transfected mouse lungs, which indicates occurence of acute lung injury. The 539-m mice showed increased weight loss compared to the NC-m mice (but there were no statistically significant differences) (Fig. 1G). In addition, 539-m-transfected mice exhibited a ten times heavier bacterial burden in their lung tissues (10^7 CFU/mg) compared to NC-m-transfected mice (10^6 CFU/mg) (Fig. 1H). Critically, our survival analysis showed that systemic delivery of 539-m significantly increased mouse mortality after PA14 infection in comparison with the control group (Fig. 1I).

Infection and inflammation are often associated with inflammasomes, cGAS, and Toll-like receptors (TLRs). Numerous published reports suggest that miRNAs are involved in regulating inflammatory responses through these pathways. We speculate that the regulatory effect of miR-539 on the inflammatory response in *P. aeruginosa* infection is also likely associated with these pathways. In general, miRNAs function through their target protein(s) that may have certain homologus sequence with the 3'-end of miRNAs. To model the potential working mechanisms, we predicted the targets of mu-miR-539–5p

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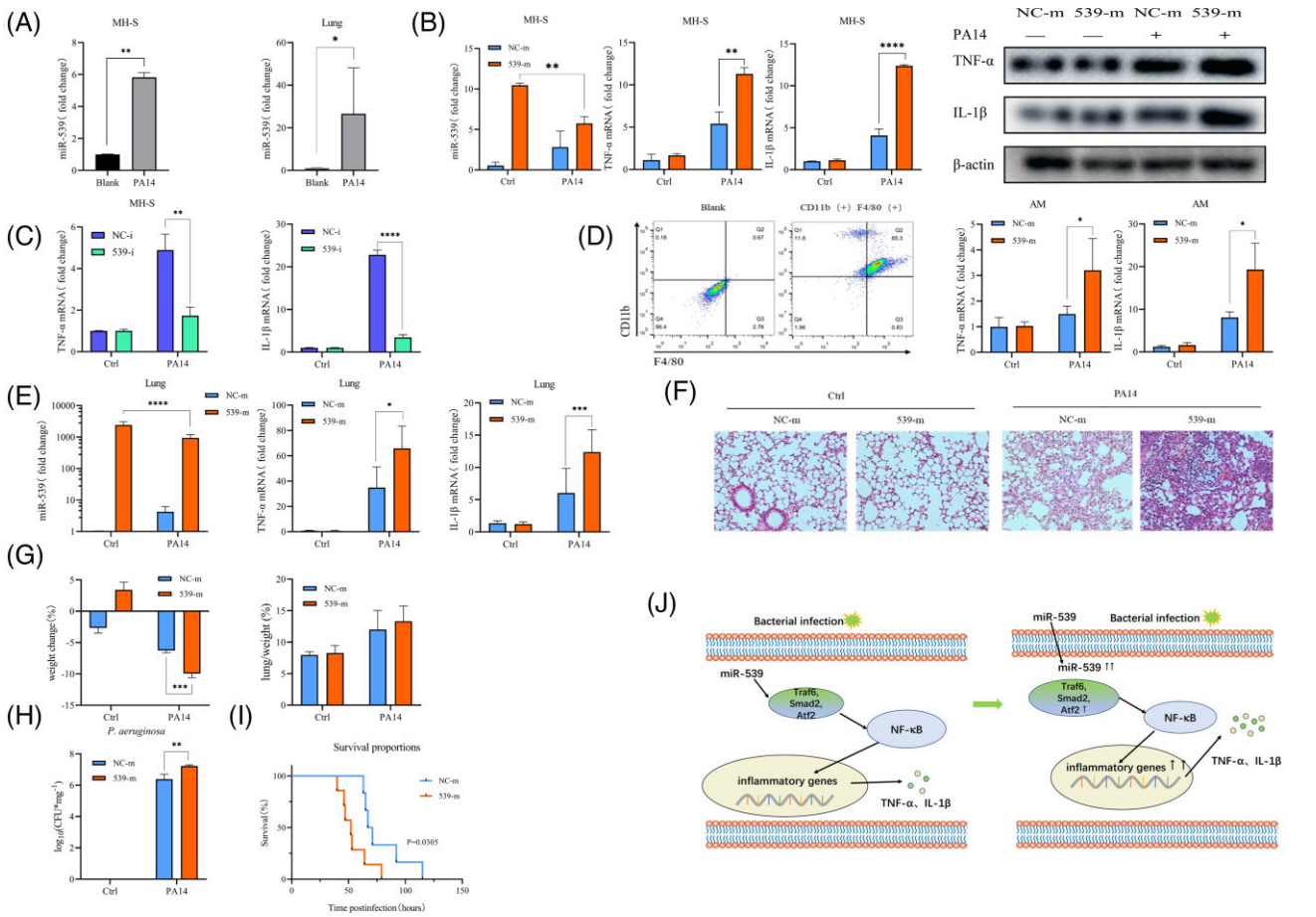

Figure 1. miR-539 is highly expressed following *P. aeruginosa* infection; and overexpression of miR-539 exacerbates *P. aeruginosa* pneumonia inflammatory responses and accelerates mortality of mice. (A) miR-539 expression in MH-S cells was determined by RT-qPCR after the cells were infected with PA14 at MOI 10:1 and 5 × 10^6 CFU in 50 μl PBS for 1 hour and 24 hours, respectively. (B, C) Expression of inflammatory cytokines in MH-S cells after inhibition or stimulation of miR-539 expression and PA14 infection. (D, E) miR-539 increased proinflammatory cytokines' production in lung tissues and AMs of *P. aeruginosa*-infected mice. (F) Lung inflammation was assessed by morphologic analysis. The lungs were embedded in formalin and sections were analyzed by hematoxylin and eosin staining. Scale bars, 50 μm. (G) Weight changes in mice before and after bacterial infection and lung tissue weight to body weight ratio after bacterial infection. (H) Bacterial burden in the lungs was assessed 24 hours after infection. (I) Mice were intravenously injected with vehicle containing either NC-m or 539-m (50 μg per mouse) 24 hours before PA14 challenge (4 × 10^5 CFU per mouse). Kaplan-Meier survival curves of PA14-infected NC-m or 539-m injected mice (n = 6, two independent experiments). Survival was determined up to 120 hours (P = 0.0305, Log-rank test). All data were presented as means ± S s.d., n = 3.(* P < 0.05, ** P < 0.01, *** P < 0.001,****P<0.0001 by Student's t-test or one-way analysis of variance with Tukey's post hoc). (J) Schematic diagram of the predicted mechanism by which miR-539 regulates inflammatory cytokines through Traf6, Smad2, and Atf2. In bacterial infection, miR-539 promotes expression and secretion of inflammatory cytokines by activating these presumed targets and regulating activation of NF-κB.

via TargetScan and miRDB, and identified *Traf6*, *Smad2*, *Atf2* and *Il1rap* in both databases (Supplementary Table 1 and Table 2). These genes have been widely reported to be involved in the regulation of inflammasomes, cGAS/STING, and TLR/NF-κB signaling.[7–10]

In conclusion, our study suggested that miR-539 induced by *P. aeruginosa* infection facilitated production of inflammatory cytokines in macrophages, exacerbating lung tissue damage and reducing the survival of the bacteria-infected mice. In addition, we predicted three targets and discussed their possible functioning mechanisms for miR-539 by analyzing the information of miRNA databases (Fig. 1J). Our study reveals a role of miR-539 in *P. aeruginosa* infection, which may be a potential target for treating related infectious diseases. Further studies are needed to identify and validate miR-539 targets and understand the underlying mechanisms.

Supplementary materials

Supplementary data is available at *PCMEDI* online.

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Author contributions

J.L., Q.Y., and M.W. conceived and designed experiments. J.L. performed most of the experiments with help from other authors and wrote the manuscript. X-H.G., F.C., and X-X.G. performed some experiments. L.C and M.W. revised the manuscript. All authors have read and approved the manuscript.

Conflict of interest

None declared. Besides, as the Editorial Board Member of *Precision Clinical Medicine*, the authors Lei Chen and Min Wu were blinded from reviewing and making decision on this manuscript.

References

1. Tacconelli E, Carrara E, Savoldi A, *et al.* Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318–27. doi:10.1016/S1473-3099(17)30753-3.
2. Zhang D, Lee H, Wang X, *et al.* A potential role of microvesicle-containing miR-223/142 in lung inflammation. *Thorax* 2019;**74**:865–874. doi:10.1136/thoraxjnl-2018-212994.
3. Zhou X, Li X, Wu M. miRNAs reshape immunity and inflammatory responses in bacterial infection. *Signal Transduct Target Ther* 2018;**3**:14. doi:10.1038/s41392-018-0006-9.
4. Zhou X, Li X, Ye Y, *et al.* MicroRNA-302b augments host defense to bacteria by regulating inflammatory responses via feedback to TLR/IRAK4 circuits. *Nat Commun* 2014;**5**:3619. doi:10.1038/ncomms4619.
5. Huang T, Pu Q, Zhou C, *et al.* MicroRNA-302/367 cluster impacts host antimicrobial defense via regulation of mitophagic response against *Pseudomonas aeruginosa* infection. *Front Immunol* 2020;**11**:569173. doi:10.3389/fimmu.2020.569173.
6. Li X, He S, Li R, etal. *Pseudomonas aeruginosa* infection augments inflammation through miR-301b repression of c-myb-mediated immune activation and infiltration. *Nat Microbiol* 2016;**1**:16132. doi:10.1038/nmicrobiol.2016.132.
7. Fledderus JO, van Thienen JV, Boon RA, *et al.* Prolonged shear stress and KLF2 suppress constitutive proinflammatory transcription through inhibition of ATF2. *Blood* 2007;**109**:4249–57. doi:10.1182/blood-2006-07-036020.
8. Yang Q, Ren GL, Wei B, *et al.* Conditional knockout of TGF-βrii/Smad2 signals protects against acute renal injury by alleviating cell necroptosis, apoptosis and inflammation. *Theranostics* 2019;**9**:8277–93. doi:10.7150/thno.35686.
9. Yi XM, Li M, Chen YD, *et al.* Reciprocal regulation of IL-33 receptor-mediated inflammatory response and pulmonary fibrosis by TRAF6 and USP38. *Proc Natl Acad Sci U S A* 2022;**119**:e2116279119. doi:10.1073/pnas.2116279119.
10. Xing Y, Yao X, Li H, *et al.* Cutting edge: TRAF6 mediates TLR/IL-1R signaling-induced nontranscriptional priming of the NLRP3 inflammasome. *J Immunol* 2017;**199**:1561–6. doi:10.4049/jimmunol.1700175.

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