Author’s response to reviews

Title: LncRNATUG1 alleviates sepsis-induced acute lung injury by targeting miR-34b-5p/GAB1

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Reviewer reports:

Varsha Suresh Kumar, PhD (Reviewer 1): The authors have done a comprehensive study on the role of TUG1 lncRNA and its downstream targets in models of sepsis induced acute lung injury. The experiments have been logically carried out. Please address the following points:

1. Could the authors please indicate in the results and figure legends (Fig2 to Fig6) how many mice/ samples were used for each of the individual experiments. The use of dot plots in place of bar graphs wherever possible would also be helpful.

Reply: Thanks for the question. The mice number used in each Figures was mentioned in the methods and figure legends section that the number of mice in each group was 12 and the lung tissues of all mice were harvested after death or at the end of the study for further analysis. To indicate the number of mice/samples in each experiment might be redundant.

As we showed three or four groups in most figures, the bar graphs (with error bars) are better for comparing the differences among groups.

2. For the Dual Luciferase reporter assay, what amount of the vectors was used for transfection.

Reply: Thanks for the question. 500 ng total DNA of the vectors was used for transfection in dual-luciferase reporter assay. This information has been added in the Methods section.

3. Were the miR mimics synthesized with a specific design/ characteristic? If so, the authors should add some details about the miR mimics synthesized by GenePharm in the methods section.
Reply: Thanks for the question. The mimics used in this study were synthesized using a standard method.

4. Could the authors please include a quantification for Fig2A.

Reply: The quantifications of TUNEL and caspase-3 staining (% of positive cells) were added in revised manuscript.

5. Study of lncRNA TUG1 expression in human lung tissue sepsis samples would further highlight the clinical relevance.

Reply: Thanks for the suggestion. However, as lung tissue samples cannot be easily obtained, we measured the TUG1 expression in the blood samples of patients with acute respiratory distress syndrome (n=15) and healthy controls (n=68). Result showed that the serum level of TUG1 in ARDS patients was significantly higher than that in healthy subjects (Fig 1D).

6. Some minor grammatical errors need correction.

Reply: The grammatical errors have been corrected in the revised manuscript.

Saroj Nepal (Reviewer 2): The manuscript "LncRNA TUG1 alleviates sepsis-induced acute lung injury by targeting miR-34b-5p/GAB1" by Qui et al has shown that TUG1 alleviates sepsis-induced inflammation and apoptosis via targeting miR-34b-5p and GAB1. The manuscript is written well and is interesting. However, I have some concerns:

1. Change in TNF-alpha and IL-6 does not seem significant. Will this change be physiologically relevant (Fig. 2D)? What is the mechanism through which mRNA levels have altered between the groups (Fig. 2C)? It would be better to discuss these aspects.

Reply: Thanks for the question. The protein levels of TNF-α, IL-1β, IL-6 in mouse lung tissues were measured using ELISA and normalized to total protein content. Our statistical analysis showed that the changes of all the three cytokines were significant between the CLP+Ad-GFP and CLP+Ad-TUG1 groups. Previous data showed that TUG1 inhibited the expressions of pro-inflammatory cytokines at both mRNA and protein levels in tissues with inflammatory injury (Overexpression of the long noncoding RNA TUG1 protects against cold-induced injury of mouse livers by inhibiting apoptosis and inflammation 2016; Overexpression of long non-coding RNA TUG1 alleviates TNF-α-induced inflammatory injury in interstitial cells of Cajal 2019). Thus, we also evaluated the effect of TUG1 on the mRNA expressions of key proinflammatory cytokines and found that the mRNA levels of TNF-α, IL-1β, IL-6 were significantly decreased in mice injected with CLP+Ad-TUG1 as compared to the CLP+Ad-GFP group. These results suggested that the change in the levels of proinflammatory cytokines could be physiological relevant. However, the mechanism of the anti-inflammatory role of TUG1 remains unclear. Future investigations will be needed to explore the involvement of TUG1 in classical inflammatory pathways.
2. The authors have used a very high dose of LPS 1 mg/ml for their experiments in vitro. What is the rationale behind this dose?

Reply: Thanks for the question. The LPS used in this study was 100 ng/mL instead of 1 mg/mL. We have corrected this error in the methods and figure legends section. We chose this dose based on the methods previously described in “Inhibition of nuclear factor of activated T cells (NFAT) c3 activation attenuates acute lung injury and pulmonary edema in murine models of sepsis 2018” and our preliminary experiments.

3. The authors have only measured pro-inflammatory cytokines. They should measure anti-inflammatory cytokines such as IL-4, IL-10 also to strengthen their conclusions.

Reply: Thanks for the suggestion. The expression levels of IL-4 and IL-10 in all groups of mice have been added as Fig. 2E. CLP operation induced the release of IL-4 but decreased the expression of IL-10 in mouse lung tissues. TUG1 overexpression significantly elevated the levels of IL-4 and IL-10 as compared to control-vector-injected mice following CLP surgery.

4. The prediction analysis needs to be shown which will help better understand why miR-34-5p was chosen as a potential target for TUG1.

Reply: The prediction analysis was performed on the website of TargetScan, which showed that miR-34b-5p was a potential target for TUG1 with a putative binding site.

5. What happens to GAB1 expression in TUG1 adenoviral vector injected group in CLP model needs to be performed. The authors directly jump into Fig. 5 and suggest GAB1 as a target of miR-34b-5p, which is not convincing.

Reply: Thanks for the suggestion. The injection of TUG1 adenoviral vector partially but significantly recovered the expression of GAB1 in CLP-treated mice. This data has been added as Fig. 5F in the revised manuscript.