Reviewer's report

Title: MicroRNA and mRNA expression profiling in rat acute respiratory distress syndrome

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Reviewer: Tamas Dolinay

Reviewer's report:

Huang et al. discuss a novel approach of discovery of gene-products that play a role in the mechanism of acute lung injury (ALI). The authors performed simultaneous microRNA (miRNA) and messenger RNA (mRNA) profiling on rat lungs that were subjected to the combined treatment surfactant depletion and mechanical ventilation. Results of gene expression were compared to animals who received no surfactant depletion (sham). Their findings suggest that there is a strong correlation between miRNA and mRNA expression changes in ALI/ARDS.

The strength of the manuscript is that it highlights the importance of post-transcriptional modification of gene expression in the mechanism of ALI. It uses a reasonable animal model and it follows a sound methodology. However in its current format the manuscript has major weaknesses and requires further work. Please see below:

Major Compulsory revisions:

1. Animal model:

A. Controls. It is not discussed what intervention is performed on the sham animals. This is an important point because the animals in the treatment group receive low, than high tidal volume ventilation, finally they undergo lavage. While this seems to be a reasonable approach to induce lung injury, it is important to know what is called sham. The authors should provide a cartoon that describes the time and length of interventions.

B. Lung injury is only expressed as histology findings. Please include at least one more form of quantitative measurement of lung injury. I recommend Evans-Blue dye extravasation studies as this will not be altered by lavage of the lungs during the experiment.

C. How do the authors measure animal well being during the experiment? A major concern is hypotension in lavaged animals, which can be a confounder that contributes to lung injury. Please describe, if animals become hypotensive during the experiment.

2. Gene expression studies:

A. While the manuscript raises the importance of posttranscriptional modification
of gene expression in ALI it fails to answer whether it is biologically important. The authors should provide biochemical evidence (co-immunohistochemistry or chemical inhibition) of miRNA targets to show that mRNA expressions change with them. Otherwise it will be hard to convince the audience that their findings contribute to changes on protein level. A potential exciting target is: let miRNA family. A good example of such work cited by the authors: Vaporidi et al. Am J Physiol Lung Cell Mol Physiol. 2012 August 1; 303(3): L199–L207.

B. It is hard to draw any meaningful conclusion from study linking miRNA to singling pathways (Fig 7). I believe the better approach would be to identify one miRNA that changes mRNA expression and analyze what are the potential other targets (genes and pathways). This could significantly elevate the relevance of this work.

Minor Essential Revisions:
1. Abstract:
   A. Line 1. ARDS is a severe form of injury.
   B. Line 4. ARDS provide insights.
   C. Line 18. The use of “dys-regulation” is very confusing throughout the manuscript because it suggests that somehow this expression change is not “well-regulated”. I would continue with the expression “regulated”. In the same sentence: rat model of VILI. ARDS is preserved for human disease.

2. Background:
   A. The first 2 paragraphs needs to be re-written because its current form is superficial and does not explain why the investigation is done.
   B. In the context of the manuscript, I would focus on introducing ARDS as a disease severe inflammation and cell damage.
   C. Please explain the role of animal modeling of ARDS (complex and heterogeneous mechanisms that are hard to discern from human samples).
   D. If the authors wish to use human outcome data, please use more recent epidemiology as ARDS morbidity and mortality has improved since the sited reference.
   E. Reference 19 is not a miRNA but mRNA-based study.
   F. The authors cite 22 classes miRNAs, but articles talk about several hundreds of miRNA (see Transl Res. 2011 April ; 157(4): 180–190). How does this compare?
   G. Paragraph 3: I would separate MRNA and miRNA profiling. In the current format it is confusing and does not help the reader to learn why it is important to perform both analyses. The key here is that they have additional value and miRNA can alter the post transcriptional level of mRNA.
   H. Last paragraph. The current content belongs to results section. The authors should discuss the goals of the study here: 1. perform simultaneous miRNA and mRNA profiling in a non-infectious model of VILI.2. Identify potential interactions
between miRNA and mRNA expression. 3. Impact of these interactions on the mechanism of ARDS.

3. Results
A. Animal model. As detailed above, Table 1 needs to include data from controls.
B. Second paragraph. References do not belong to the Results section. It should be moved to the Discussion.
C. MiRNA and mRNA profiles section: Why did the authors use a different threshold for the miRNA array results than for the mRNA? If this was because of loosening the criteria to find more genes, the authors should discuss it in the Methods section.

4. Methods
A. Please explain the abbreviation SAM and provide reference.
B. Bioinformatics analysis. Since the manuscript heavily relies on available software package a brief description why the particular package was used is warranted.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I no competing interests.