Reviewer's report

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

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Reviewer: Maneesh Bhargava

Reviewer's report:

This is a well-written manuscript in an important area of research. Systems level approach for identifying mechanisms that are involved in pathogenesis of ARDS will improve our understanding of this disease and also result in targets for therapy. However, I have several concerns about this manuscript

Major Concern

1. What are the controls that are shown in Figure 1? Were these rats with ventilation by normal tidal volume or rats that were maintained at room air? Ideally room air controls rats and mechanically ventilated rats should be included in the study.

2. Please describe how the rat lungs were fixed using formalin. Was formalin injected into the trachea with a plunger or was certain amount of pressure used for instillation into the lung? In Table 1, for hyaline membranes, the median value for ARDS is outside the range. The mean value is also below the minimum value. This is not statistically possible. How many rats were used to generate this table. Please provide statistics that compare the means and/or medians in the two comparison groups. I would also favor using standard deviation to demonstrate the variance instead of SEM.

3. Please provide wet to dry lung weight in the groups compared.


5. Purity of RNA isolation is marginal.

6. Is the mi RNA microarray modified since its development? Similarly what was the reason to choose inhouse printed DNA microarray

7. What are the backgrounds used for the DAVID GO enrichment analysis? This is very important the null hypothesis test implements hypergeometric distribution, which is sensitive to the background or 'N', used for the analysis.

8. On table 4 and 5 list the genes that mapped to the various GO annotations. If the purpose was to determine the GO terms why are PIR and SEQ mapping shown in Table 4 and 5. Typically GO has each gene has only 3 annotations-biological process, cell compartment and molecular function.

9. Show the genes that were mapped to the individual annotation term in DAVID.

10. The statistical cutoff is a major concern with methods. For Functional
annotation clustering in DAVID, several clusters with enrichment score of < 1.3 are shown. By the DAVID algorithm enrichment > 1.3 should be used for a cluster to be statistically significant. This will be even more relevant depending on the appropriateness of the 'universe of genes' for the background. Also for figure 5, an alpha of 0.05 is not appropriate as multiple comparisons are done. It is not clear if any correction is used for multiple hypothesis testing. What are the statistical cutoffs for miRpath analysis?

11. Was the functional annotation clustering done with default parameters or with modifications? What was the stringency used for the clustering?

12. Why use targetscan and miRanda

13. Text and table 6 for Let does not match

14. In context of Berlin definition of ARDS, it is best to avoid using the term 'Acute Lung Injury'

15. Please include a paragraph regarding the limitations of this study

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

No