Reviewer’s report

Title: Systems biology of interstitial lung diseases: integration of mRNA and microRNA changes

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Reviewer: Jiri Zavadil

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The study by Cho et al comprehensively summarizes changes in mRNA and miRNA levels in primary interstitial lung disease (ILD) patient samples, and by applying a powerful and elegant set of bioinformatics computational approaches the study integrates these findings into predictive models of gene regulation events underlying ILD. The questions posed in the study are defined well, the methods (both in the profiling and bioinformatics areas) are appropriate and utilized richly, the data are sound and supported by stringent statistical evaluations and the interpretation of the complex integrated data is conceptually very strong. The MS is written very clearly, other people’s work cited properly, although some difficulties in navigating the MS arise probably after editing changes that introduced discrepancies in references to figures and supplemental material. Some conclusions on key findings tend to be speculative and should be supported by additional (simple) validation experiments, suggested in the comments below.

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

1. While the study effectively defines genome-wide predictive and correlative networks of gene regulation and applies quite powerful and thorough bioinformatics processing of the data to integrate and interpret the profiling results, the “systems biology” term in the title and possibly throughout the text should be used with caution - using solely the combined mRNA and miRNA profiling poses a limit to what extent the molecular events affecting a complex disease can be interpreted.

2. Authors might want to pool Fig 1 and 2 into one composite image and change the heat map colors, see another comment in the section of compulsory revisions.

3. It would be useful for the readers to evaluate typical examples of the discussed epithelial vs mesenchymal morphologies of the MDCK transfectants – can these be shown?

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. I could not find legends for Figs 8-12, but these seem to be parts of different figures anyhow – see below.
2. Suppl files numbering is confusing compared to the references to the text – probably due to MS editing changes? E.g. Suppl file 4 refers to Suppl Fig 3 etc. Also, Fig 6A in text labeled as Fig 7 and 6B in text as Fig 8? PLEASE CONSOLIDATE THOROUGHLY.

3. Annotations on most cytoscape network images frequently unreadable, they can thus suffer a loss of informative value especially those that are meant for print

4. Pg 10 – miR-22* listed yet miR-21* shown in SF 5 (actually labeled as SF 6)

5. Pg 15 – Correct Tgfbr1 instead Tgfr1

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. If technically at all possible, change Red/Green coloring of heatmaps and networks to Red/Blue or Yellow/Blue – for rationale, see Nature Structural & Molecular Biology 14, 173 (2007).

2. Suppl Fig 3 – legend insufficient, graph rather inconclusive as bar graphs with SD(?), show as scatter plot (optionally log2 transformed) of individual measurements with mean+/SEM and run statistical analysis between genders, if you think this finding adds to the study’s conclusions.

3. One finding is of potentially high interest for the field of EMT role in fibrogenesis – the putative activation of miR-23a cluster by Zeb1 leading to downregulation of Nedd4l and stabilization of TGFb signal, correlated with the epithelial vs mesenchymal phenotype of the cells. However, the authors speculate based on induced expression level correlations that Zeb1 regulates the miR-23a cluster at the transcriptional level, and in the discussion cite unspecified TFBS analysis of miR-23a cluster regulatory regions, finding putative Zeb1 binding sites – what are these? E-box elements? And how is this regulatory region defined – from the genome data at public databases (UCSC GB) it is not very clear …

4. Two simple experiments are warranted to link these correlative findings into a well-defined novel pathway resulting from the powerful integrative approach – 1) to show that Zeb1 either directly or indirectly regulates miR-23a (either qPCR-ChIP or luciferase reporter assay on the miR-23a regulatory sequences) and 2) to show that miR-23a indeed regulates Nedd4L (a standard 3’UTR-luc assay can be implemented).

5. Some discussion would also be useful – especially when Zeb1 is found to bind to the pri-miR-23 promoter - on why would Zeb1 binding rather counter-intuitively lead to the miR-23a cluster upregulation, as Zeb1 is a known repressor of transcription, as established by its negative targeting of e.g. the promoters of E-cadherin and the miR-200 cluster…

Level of interest: An article of importance in its field

Quality of written English: Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests