Reviewer’s report

Title: Epithelial-mesenchymal transition markers screened in a cell-based model and validated in lung adenocarcinoma

Version: 0 Date: 07 May 2019

Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

No - there are minor issues

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?

No - there are minor issues

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

No - there are major issues

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?

Maybe - with major revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: The problem identified in this work is real. It is indeed a challenge that the patient-derived observations are not exactly replicated in laboratory model systems.
EMT is indeed important for cancer progression but we are still not any closer to exploit this in clinics.

Introduction is little too long and not very focused. It wanders from one topic to another and does not appear very coherent.

My one concern is the model system itself. Conditioned medium from A549 cells was used to generate CAFs and then the conditioned medium from CAFs was used to induce EMT in A549 cells. Are the results reproducible in other lung cancer cells?

Clearly, authors are trying to present A549-CAF system as the best system to study EMT - but this is not even mentioned in Abstract?

Methods section of Abstract is too inadequate. Did authors only conduct RNA seq and nothing else?

Conclusions described in Abstract are too over-reaching and do not truly reflect on results described in manuscript.

Figure 1A - while at 24 h, I can see elongation of cells in CAF group, there does not seem to be much difference at 72 h? Further, there seem to be more cells in Control group - does EMT inhibit proliferation? Also, Figure 1B needs a quantitative data. Finally, authors mention 3 and 6 hours while describing results, but only show 3 hours in the Figure.

Figure 2 - my major concern is why EMT is not evident at 72 hours? EMT markers are not statistically significantly different at 72 hours. Does that mean there is no EMT at 72 hours? This does not go well with authors own description of Fig 1 results where they claim EMT at 72 hours (even though I do not see it - see my comments above). Is CAF-mediated EMT not sustained ? In such a case, is the system even reliable?

Authors have tested several EMT markers. I would suggest including ZEBs as well. miR-3613 effects on EGFR pathway are not adequately described. Were miR-3613 levels manipulated in cells with appropriate transfections before looking at EGFR pathway genes? In fact, even though authors describe in Results that miR-3613 was the most differentially expressed, they do not show any such data where a number of top miRNAs are presented for direct comparison! Is DEmiRNA same as miR-3613 Or is it some measure of all the differentially expressed miRNAs? In summary, the miRNA results are not very convincing, mechanistically speaking.

The bioinformatics data is interesting but somehow there is no clear message. IS it possible to summarize main findings in a cartoon figure i.e. what novel information did we learn - what are the early vs late genes in EMT induction?

Finally, the study started with a premise that there is gap between in vitro systems and the clinical observations. It is not clear how this work bridged that gap. Isn't this yet another in vitro model which might still have discrepancies with clinical data? More robust correlation with TCGA data needs to be shown.
REQUESTED REVISIONS:

Please see my detailed comments above regarding my concerns.

Note: This reviewer report can be downloaded - see attached pdf file.

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**

If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**

If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**

Please indicate the quality of language in the manuscript:

Acceptable

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