Author's response to reviews

Title: MicroRNA-34a modulates genes involved in cellular motility and oxidative phosphorylation in neural precursors derived from human umbilical cord mesenchymal stem cells

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Author's response to reviews: see over
Title: “MicroRNA-34a modulates genes involved in cellular motility and oxidative phosphorylation in neural precursors derived from human umbilical cord mesenchymal stem cells”

Comments to be passed to the authors:
In the Introduction, for the statement, “MiRNAs have been shown to regulate cancer and developmental processes, such as stem cell self-renewal, neuronal differentiation, cell motility, and cell proliferation [14, 15].” Please add some updated references, e.g.

Thank you for the suggestion. We have updated the Introduction as suggested (2nd Paragraph, Page 6).

Reviewer: 1
The information in the manuscript is compelling while comparing miRNA with the development of MSCs to neural cells.
It will be important, in Figure 1A, to add protein analyses to the PCR. Perhaps imaging will suffice.
We thank the reviewer for the supporting comments. We have included immunoflorescent assay (IFA) images into the revised Figure 1A.
Reviewer: 2
In this manuscript, the authors examine the differentiation of Wharton’s Jelly Matrix mesenchymal stem cells (WJ-MSCs) into neuronal cells, and identify changes in miRNA during the process. They identify a number of miRNA differentially expressed, and, in particular, they find miR-34a and miR-206 that were previously shown to be linked to bone marrow mesenchymal stem cell neurogenesis. Interestingly, overexpression of miR-34a led to the downregulation of 136 neuronal progenitor genes that were associated with multiple functions such as cell motility, energy production and actin cytoskeleton organization. Functionally, the authors show that knocking down miR-34a resulted in increased WJ-MSC motility. The manuscript is interesting and thorough. I would recommend the following improvements:

We thank the reviewer for the supporting comments.

Major Compulsory Revisions
1) In the abstract, the methods section should contain more details.

We have included more method details into the revised abstract.

2) The microarray experiment (following overexpression of miR-34a) will only identify genes that are regulated at the mRNA stability levels by miR-34a and not the genes that are regulated through changes in translation. This should be emphasized in more details in the discussion.

Yes microarray technology can only detect RNA alternations but not the actual protein levels. We have pointed this out in the revised Discussion (1st Paragraph, Page 17)

3) In the motility assay, is it possible that part of the effect is due an increase in survival of the cells during the assay? The authors should make sure that miR-34a knockdown does not affect cell survival. Also the assay should be done at multiple time points (as opposed to only 6 hours) so that we can get a better idea of the kinetics. Finally, the motility assay should contain all the different transfected cells (si34a, scrambled, si-GFP, mock) as additional controls.
We have re-checked cell survival rate by Trypan blue staining after miR34a knockdown. No significant cell viability was affected (see above). Thank you for the reminding. We have mentioned this in the revised manuscript (Page 15, last sentence).

The motility assay was repeated at another time point (24h posttransfection). The trend that miR-34a regulates MSC motility is still preserved. We also overexpressed miR34a into cells to show that miR34a can repress cellular motility. We have included this data in the revised Fig.4C-D.

4) The discussion is a bit weak and short and should be improved. For example a discussion of the known roles of miR-34a in cell cycle/apoptosis in cancer, and how this may be related to neurogenesis would be helpful.

Thank you for the suggestion. We have modified the Discussion as suggested (Page 18).

Minor Essential Revisions
1) The current conclusion section is actually material that should be in the discussion. A new conclusion should be written that summarizes the findings, point out the new findings of this paper, and discusses their relevance.
Thank you for the suggestion. We have modified the Conclusion section as suggested.

2) What are the p values for the graphs on fig 2A?

   The p value is 0.022 for miR-34a and 0.034 for miR-17-5p.

3) Fig 3B should be improved. Would there be a way to include a better figure for these pathways?

   We have changed the way of presentation for Figure 3B. This figure is clearer.

4) the manuscript as a whole should be edited to improved the English (many typos and grammatical errors)

   Thank you for your reminding and we apology for typos. We have now rechecked the manuscript.