Reviewer's report

Title: miR-124a and miR-137 inhibit proliferation of GBM cells and induce differentiation of tumor stem cells

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Reviewer: Lucia Di Marcotullio

Reviewer's report:

General
The manuscript by Silber et al., “miR-124a and miR137 inhibit proliferation of GBM cells and induce differentiation of tumor stem cells”, reports the expression analysis of 192 miRs in human non-neoplastic brain tissues, anaplastic astrocytomas (AA) and glioblastoma multiforme (GBM). The study reveals a global decrease of miR expression in AA and GBM relative to control tissues, with 6 miRs specifically down-regulated in both tumors. Using adult neural stem cells that differentiate in response to growth factors (GFs) depletion, the authors show that two miRs, 124a and 137, are upregulated when GFs were withdrawn, and that overexpression of these miRs induces neuronal differentiation of these cells, as well as, of oligodendroglialoma- and GBM-stem cells, and inhibits proliferation of GBM cell lines. Further, the authors show that both miR-124a and miR-137 inhibit expression of CDK6 and pRB phosphorylation, suggesting that these miRs may be useful therapeutic agents for the treatment of GBM.

The presented data are convincing and support the authors' conclusions. However, the following points still need to be addressed:

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

- In figure 1B is indicated differentiation after growth factors deprivation, which induces miR 124a e 137 expression. Is it possible to obtain more detailed informations on the cell type induced, by means of specific markers?

- In figure 2A-2B, I would recommend to show a field with more cells, and present the corresponding phase contrast field, which could show the neuritic outgrowth and better represent the morphologic changes induced by miR-137. In figure 2A neuritic-like structures do not seem to be produced. I also suggest using a different marker of neuronal differentiation in addition to Tuj1, since a reduction of GFAP is not evident (figure 2A e 2B; figure 3A).

- Figure 3A quality is poor, downregulation of GFAP is not convincing; there is neither synergic effect, nor summatory effect between the two miRs, this is not further discussed, so I suggest to omit the cotransfection data (figure 3B).

- Although authors show in supplementary information the functionality of miR expressing vectors, they should also show the levels of miRNA expression by
northern blot or Q-PCR 48h and 72h after transfection, and especially 10 days after transfection as described in experiment in figure 3C.

- GBM cell lines undergo growth arrest (figure 4A-4B), what about differentiation? I would expect to see differentiation at 72h or later. Did the authors prolong the experiments for more days? This should be shown.

- Did the authors compare the relative levels of miRs overexpression? Could the fact that miR-124a reduces phosphorylation of RB more efficiently than miR-137 (figure 5B) be due to a stronger expression and higher levels of 124a? If so, I would remove the sentence on line1, page 17.

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

Discretionary Revisions (which the author can choose to ignore)

**Which journal?:** Appropriate or potentially appropriate for BMC Medicine: an article of importance in its field

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests