Reviewer's report

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

**Version**: 1, **Date**: 29 October 2007

**Reviewer**: Maria Giulia Farace

**Reviewer's report**:

**General**
This manuscript examines the role of miR-124a and miR-137 in brain tumors and in brain tumor stem cells. The authors present evidence that miR-124a and miR-137 are down-regulated in human glioblastoma and astrocytoma and up-regulated during adult neuronal stem cell differentiation. They went on performing differentiation experiments of adult neuronal stem cells and brain tumor stem cells after miR-124a or miR-137 transfection. They claim that the two miRNAs promote neural differentiation of adult neuronal and brain tumor stem cells. Conversely, the transfection of miR-124a or miR-137 resulted in inhibition of glioblastoma cell line proliferation. Although the authors present an interesting hypothesis on the mechanisms by which miR-124 and miR-137 may regulate neuronal differentiation or cell proliferation, there are a number of issues discussed below, that need to be resolved.

----------------------------------------------------------------------------------------------------------------------

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

1) In the differentiation experiments of SVZ NSCs, performed by simply removing mitogens from the culture medium and shown in Supplementary Figure 3, a co-staining or at least a merging of GFAP and Tuj1 images from the same fields would greatly improve the quality of the data. Without this it is impossible to appreciate which percentage of cells are positive for each marker. It is in fact well known that mere growth factor withdrawal usually induces a great majority of GFAP+ cells, and only a small portion of Tuj1+ cells. These numbers for this experiment are needed in order to evaluate the significance of the concomitant miRNA upregulation in correlation with neuron-like differentiation. Moreover, the morphology of the cells induced to differentiate by this method and shown in suppl. Fig. 3 could be very difficultly defined as neuron-or astrocyte-like, as should instead be expected by standard protocols of mNSCs differentiation.

2) In the experiments testing the ability of miR-124 and miR-137 to promote neuronal differentiation of mNSCs, authors claim that the transfection efficiency was about 80-90%, but they do not demonstrate that the transfected cells are the same that are pushed to differentiate. A control transfection with a labelled
miRNA mimic would clearly demonstrate the coincidence between miR-transfected and neuronally differentiated cells, and might probably provide an explanation for the difference between declared transfection efficiency (80-90%) and the observed increase in Tuj1+ cells (5-fold). Moreover, it is not clear why the authors stopped the differentiation experiment after only 72 hours, while with other cells (e.g. GBM CD133+ cells) the differentiation protocol was continued for 7-10 days, as described in most of the published protocols. Another way to more convincingly demonstrate a correlation between miR-124a and miR-137 expression and differentiation would be to assay the effects of a specific inhibition of each or both miRNAs through LNA antisense oligonucleotides. In this way, the importance of miR-124a and miR-137 upregulation with respect to differentiation would be more clearly estimated.

3) In experiments dealing with GBM stem cells, the major drawback is that only one sample is employed. Moreover, while the results in CD133+ cells are in fact in favour of a pro-Tuj1+ differentiation by both miR-124a and miR-137 in the absence of growth factors, in CD133- cells the effect of miR-124a and/or miR-137 on marker expression is apparently indistinguishable from that of the exposure to growth factors. How do authors explain this?

4) Regarding the regulatory relationship linking miR-124a, miR-137 and CDK6, no evidence of a direct action of these two microRNAs onto CDK6 mRNA is provided. A direct demonstration of the presence of miR-124a- and miR-137 responsive sites in CDK6 3'UTR is absolutely necessary before drawing any conclusion. Moreover, in the paper cited in reference n. 25, where CDK6 was established as a target for miR-124a, no regulation of mRNA for CDK6 was observed, and the action of miR-124a on CDK6 was limited only to the protein level. In the present work, on the contrary, the strong reduction of CDK6 transcript might account for a different way of modulation, probably indirect and mediated by some transcriptional event. Again, a luciferase reporter assay would probably clarify this point.

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) The conclusions drawn in HGA (AA + GBM) about miRNA modulation derive from the analysis of a small number of samples (i.e. 4 AA + 4 GBM), too limited to yield data with a clinical relevance. Moreover, the control samples used for this assay were chosen among gliosis cases that, although being of course non-tumoral, are subjected to pathologic processes that might somehow impair the results obtained. Other papers cited by the authors (ref 20 and 21) in fact used different tissues as non-tumor controls, such as tumor-free areas from the brains of glioblastoma patients, and not only, as authors claim in the Discussion section, normal human fetal and adult brain.

2) About the epigenetic regulation of miR-137, authors claim that figure 1C demonstrates that the combination of 5azadC + TSA upregulates miR-137 expression more strongly than 5azadC alone. The values depicted by the bars (+/- SD) in figure 1C do not seem to support this conclusion, just like those
shown in supplementary figure 5, neither in U87 nor in U251. The variations shown have no apparent statistical significance.

3) Regarding the differentiation power of miR-124a and miR-137 on mOSCs, it is not clear why authors assess and discuss the ability of miR-124a to reduce the numbers of GFAP+ cells, but they do not say that also miR-137 can do it to a very similar extent (see fig. 3B). Moreover, in the figure describing these results, fig. 3B, the use of the same scale on the y axis for both Tuj1 and GFAP values would provide a better overall picture of the respective proportions of labelled cells in all conditions.

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