Reviewer's report

Title: The Effects of MicroRNA Transfections on Global Patterns of Gene Expression in Ovarian Cancer Cells are Functionally Coordinated

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Reviewer: Kotb Abdelmohsen

Reviewer's report:

The manuscript by Shahab et al, aimed to understand the functional consequences of miR-7 and miR-128 overexpression in Hey ovarian cancer line. Authors relied on gene arrays and available algorithms to understand and explain the effects of these two miRNAs. Both miR-7 and miR-128 is known to regulate EGFR expression and thus they used this link in an attempt to gain more information and examine whether it mediates the effects of these miRNAs.

The reviewer finds this manuscript is rather premature and confusing for the following reasons.

1) The manuscript heavily relies on microarrays after overexpressing miR-7 and miR-128. This represents only the changes in steady state levels of mRNAs.

2) miRNAs are also known influence mRNA translation which is completely ignored in this manuscript and will not be revealed by microarrays.

3) Why authors decided to investigate these two miRNAs and no other miRNAs? I find the use of these two miRNAs is not justified.

4) Why do we need to compare the effects of these miRNAs on gene expression? They have different seed sequences and do not have anything in common except that they target EGFR mRNA which might be targeted by many other miRNAs.

5) Authors heavily rely on algorithmic predictions for data analysis.

Major Compulsory Revisions

1) The data shown in Figure 1 is not relevant to the manuscript since these miRNAs are known to regulate EGFR mRNA.

2) Page 3 last 2 lines. Authors concluded that miRNAs may be clinically useful in cancer therapy. What is new in this?

3) Data obtained by global microarray analysis must be validated by RT-qPCR.

4) miR-induced changes are different from the changes induced by EGFR downregulation. This is somehow expected since miRNAs are fine-tuning gene expression with much broader effects than EGFR-siRNA. Again this comparison was also based on microarray analysis which ignored the possible effects on mRNA translation.

5) The data in pages 6 and 7 are very confusing. Authors identified upregulated
genes after miR-128 overexpression. Then they wanted to test the hypothesis that endogenous miRNA are out competed by the exogenous miR-128. Out competing may not means changes in the expression. Again they used predictions (Genomica) to find miRNAs that could potentially target these upregulated genes and identified 78 miRNAs. Experimental evidence is required while assessing the levels of miRNAs before or even after miRNA transfection may not be appropriate to explain upregulated genes after miR-128 overexpression.

6) The effects of these miRNAs on cell adhesion and cell cycle are not thoroughly performed. Authors need to provide evidence for cell cycle arrest by these miRNAs. The effect of miR-7 on cell cycle is more significant than miR-128. It would be great to Western blot analyses of some of the cyclins for example to support the FACS data. miR-128 does not seem to have a great impact on cell cycle, it might have a minor effect. It would be great to confirm by Western bloting.

7) What is the cell number after transfecting miR-7 or miR-128?

8) Cells transfected with miR-7 show less cell population in S-phase. Do these cells have less DNA replication? What is the effect of miR-128 on DNA replication? From Figure 5B, it seems they might have opposite effects on DNA replication as seen in the S-phase.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

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.