Peer Review File

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Review Comments

In the manuscript "MiRNA 3613-5p and miRNA 3916 rescued the inhibition of cell migration in CNOT2 depleted MDA-MD-231 cells" the authors present data on siRNA mediated knockdown of CNOT2, a subunit of the CCR4-NOT complex, which leads to both downregulation of the miRNA-biogenesis related genes Dicer and DGCR8, as well as differential expression of different microRNAs using microarray. Some of the miRNAs are validated using qPCR, and rescue experiments performed using a cell migration assay with one of the siRNAs. Attached you find my comments that keep me from accepting the manuscript in its current form:

Major points:

1-First of all the manuscript is filled with typos, misspellings (e.g., rescured instead of rescued), double spaces, and also missing spaces. The quality of the English writing should be revised (e.g., "the CNOT" sounds odd, either "the CNOT complex" or "CNOT" alone).

(Response) Thank you. Corrected

2-Throughout the manuscript (including tables and figures) the authors do not use the appropriate annotations for microRNAs, hsa-mir-xxx-xp should be used for all miRNAs, especially in the supplemental file the star annotation that are outdated since years should not be used.

(Response) Corrected.

3- The description of introduction and discussion, but more importantly the methods are very limited. Information has to be extended here (e.g., which microarrays used,

manufacturer; RNA interference probes, manufacturer, sequence; parameters for DAVID analysis).

(Response) Thanks. Added.

4-The statistical analysis applied is wrong for the data from Figure 1, this should be an ANOVA as three groups are used (provided that the data are normally distributed, if not non-parametric testing has to be used). Using a t-test repetitively is not correct! Also, SEMs should be displayed instead of SDs and n-numbers provided.

(Response) Thank you for your comment. Corrected.

5-For the wound healing assay it is not stated which of the two siRNA probes was used. What do the authors mean with positive and negative? More detailed information has to be provided for these experiments in both the methods and the results.

(Response) Thank you for your comment. Instead of siRNA, we used stable shRNA. Therefore, information was added. Negative and positive control indicate scratch before and after cell migration.

6-Why were Dicer and DGCR8 chosen as possible genes affected by CNOT2 knockdown? What about other genes involved in miRNA biogenesis pathways?

(Response) Thank you for your excellent comments. In the future study, we are going to plan to see other genes involved in miRNA biogenesis by interacting with CNOT2.

7- The GW182 should be replaced by TNRC6A, or this gene name should at least be added.

(Response) Added.

8-qPCR primers should be listed in a table for easier comprehension.

(Response) Table for PCR primers was added.

9-A general caveat is that microarrays have been used, which compared to miRNA sequencing only have lower sensitivity and do not allow for quantification of miRNAs. And no heat maps have been supplied for the microarray data.

(Response) Thank you for your comment. We agreed on your comments. In the further study, we are going to carry out deeper analysis.

10- Figure 2a: the authors write that "red indicates upregulated miRNAs, blue indicates downregulation miRNAs", there is no red; mirna names are not approriate, spell checks (red underlining) is still included in the table).

(Response) Thanks. Corrected.

11-Figure 2b: y-axis: what do the values represent? is this relative level / U6? shouldn't controls be 1.0 then?

(Response) Thank you for your comment. Y axis represents relative expression of miRNAs. Delta Ct for my control group (non-treated) normalized to the endogenous control as being equal to 1

12-Validation of differentially expressed genes: why were the 4 miRNAs used? mir7 and mir10a are not even included in the top 10, no downregulated miRNAs have been validated. Why is this?

(Response) Thank you for your comments. We randomly selected the miRNAs for validation.

13- Figure 3: the KEGG pathway numbers used are wrong: Wnt signaling pathway would be KEGG: 04310 (or hsa04310) instead of p00057; the x-axis is not labelled; what is the axis representing? -log10 p value?

(Response) I am sorry to make you confused. We performed PANTHER pathway instead of KEGG.

14-Pathway analysis: how about GO annotations, which genes are targeted by the diff expressed miRNAs? Are there any hub genes involved? Do those deregulated miRNAs target any miRNA biogenesis pathway genes? A deeper analysis should be provided. And the spell check mark ups are again in the figure.

(Response) Thank you for your comment. We agreed on your comments. In the further study, we are going to carry out deeper analysis.

15- Figure 4: the migration assays should be quantified as, although the pictures are convincing no conclusions can be derived without statistical analysis.

(Response) Thank you for your comments. Quantification for the migration assay was added

16- Supplementary: the lists are not sorted descendingly; fold changes should be provided as negative fold change, the values between 0-1 are not helpful; the correct miRNA annotations should be used. The color legend should be explained at least somewhere.

(Response) Thank you for your comments. We selected fold change cut-off (fold change≥ 2, p value < 0.05) for the supplementary data. The color legend was explained.