

## OPEN PEER REVIEW REPORT 1

**Name of journal:** Neural Regeneration Research

**Manuscript NO:** NRR-D-19-00327

**Title:** The Role of MiR-124 in the Regulation of Retinoic Acid-induced N2a Cells Differentiation

**Reviewer's Name:** Jigar Pravinchandra Modi

**Reviewer's country:** USA

**Date sent for review:** 2019-06-18

### COMMENTS TO AUTHORS

The authors' findings about "The Role of MiR-124 in the Regulation of Retinoic Acid-induced N2a Cells Differentiation" are very interesting; However, there are some concerns regarding preparation of this manuscript as follows:

Major revisions:

1. Author must provide list of Abbreviations that all are used in manuscript for readers. It is difficult to follow short forms in manuscript if full list of Abbreviations is not available. Example on Page 8, line 50, Full form of PTBP1?

2. In methods section, on page 3, line no 53 stated that equal amount of DMSO in DMEM. Equal amount to what? What is the concentration of DMSO used by author in experiment in control cells?

3. In results section. Please highlight the subheadings and provide regular no to them also.

Author must mention the RA dose 10 or 20  $\mu$ M used in miRNA and miR-124 experiments.

- On Page 7, line 1 mention that miR-124i, inhibitor negative control (iNC), miR-124 or negative control (NC) 48 h after treatment. What treatment? Please rewrite that sentence which is very confusing.

- On Page 7, line 25, Be consistent with using short form. Negative control = NC than used that form in manuscript. Don't change is to nc.

- On Page 7, line 28; To conform the results sounds improper so change it to confirm the results.

4. Discussion is very poorly written; however, some paragraphs from the Discussion would better fit into the Introduction. "MiR-124 is expressed mainly in central neural system both in embryo and adult (Lagos-Quintana et al., 2002; Kim et al., 2004; Aboobaker et al., 2005; Wienholds et al., 2005). It is the most abundant miRNA expressed in the brain (Lagos-Quintana et al., 2002).

- In Discussion section please remove (Figure 2) on page 7 and on page 8 (Figure 3-5) from the manuscript.

- Some of the sentences from discussion must remove from discussion and put it in methods and results section. Those are mentioned below

a. MiRDB, TargetMiner and TargetScan were used to predict the targets of miR-124.

b. The common targets predicted by the three kinds of software were subject to GO analysis.

c. Genes with GO\_0030182 (neuronal differentiation) might be the targets of miR-124 in RA-induced neuronal differentiation.

d. We got a list of 116 genes by this way (Data not shown).

e. Here, miR-124, miR-125b, miR-9 and let-7a expression changes during RA induced N2a cells differentiation were measured by Real-time PCR.

- Discuss the results properly in discussion section, and don't repeat the results as you mentioned in start of discussion like "The results showed that miR-124 and miR-9 were up-regulated while miR-125b expression decreased".

5. In Figure 1D, Surprising after 24 hr differentiate growth of Na2 cells is decreasing in 48 hour and it becomes doubles at 72 hour with RA treatment.

Author must repeat cell experiments to confirm this result again because N=3 is not enough to tell that difference. Because we see day dependence differentiation after 48 hours, so what is the reason that initial increase (24 hour) in differentiation is decreased at 48 hours?