Reviewer's report

Title: Deep Sequencing the microRNA profile in rhabdomyosarcoma reveals down-regulation of miR-378 family members.

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Reviewer: steven cheng

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This is an interesting work in which the authors conducted deep sequencing analysis of miRNAs in a cohort of rhabdomyosarcoma samples and identified that the miR-378 cluster was drastically downregulated. Subsequent experiments showed that miR-378 promotes apoptosis of the embryonal form of RMS cell line, RH30, when over-expressed therein. However, there are a number of important deficiencies that prevented me from making a favorable recommendation. The major compulsory issues are as follows.

1. The authors claimed in the Introduction that members of the entire miR-378 cluster were “strongly under-represented in ARMS and ERMS samples. However, the data are not clearly presented to substantiate this point. Also, are these miRNAs produced in a polycistronic transcription unit or scattered in different chromosomal loci? If the latter is the case, are those separate genes regulated differently?

2. The authors also claimed that miR-378 targets IGF1 and presented one Western blot to support that (Fig. 2B). It is not clear if this regulation is direct or indirect? Is there miR-378 recognition sequences in IGF1 3’UTR, coding region? An experiment using luciferase reporter construct with IGF1 3’UTR is necessary to address this issue.

3. The miR-378 cluster was identified from a cohort primary RMS samples consisting of both ARMS and ERMS types. However, the experimental testing was done only in the embryonal type RH30 cells. It is necessary to repeat the testing with additional RMS cell lines, particularly the ARMS type.

4. The authors showed that over-expression of miR-378 induced a number of myogenic markers, implying that it has a function of promoting myogenic differentiation of RH30 cells. However, the experiment supporting that claim was only the transient transfection. The proper way to make that point is to induce myogenic differentiation in vitro without or with ectopically expressed miR-378 in RH30 and other RMS cells.

5. The 5’-Aza experiments were confusing and seemed to be irrelevant, since miR-378 promoter (which one?) was not methylated. This observation suggests that the observed 5’-Aza effect might have been indirect, despite it induced miR-378 expression.

6. The authors neglected to cite several important recent articles on miRNA in rhabdomyosarcoma. Please cite Huang et al, MiR-214 and N-ras regulatory loop

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**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