Reviewer's report

Title: MicroRNA-182 Promotes Metastasis of Hepatocellular Carcinoma Through the Down-regulation of Metastasis Suppressor 1

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Reviewer: Paolo Gandellini

Reviewer's report:

Wang et al. investigated the potential of miR-182 as an oncogenic miRNA involved in HCC progression and validated MTSS1 as a miR-182 target. The study includes characterization of clinical samples and in vitro investigations using cell cultures for manipulation of miR-182 expression. The findings are interesting but some aspects need to be further elucidated before the study may be suitable for publication. Overall, the manuscript requires language correction to avoid confusion in some parts.

Major compulsory revisions

• Figure 1. Please specify how positivity rate for MTSS1 (IHC staining) was determined on tissue sections. Do the percentages represent the average percentage of positive cells across tumor and normal samples? If so, standard deviations should be reported in Fig 1G. Alternatively do they represent the % of positive samples? If this is the case, which was the cut-off used to discriminate positive vs negative samples?

• Figure 2A: it would be informative to report the relative expression of miR-182 for all normal tissue/tumor pairs. This would actually add valuable information about the distribution of miR-182 expression across samples.

• Figure 2B: average expression of miR-182 in normal tissues should be reported in the same graph (together with the average expression in tumors), to make it possible to compare normal samples with tumors of different grades. In addition, relative expression levels reported in the figure legend do not correspond to what reported in the graph.

• Figure 2C-D: showing western blotting of MTSS1 and qRT-PCR for miR-182 is not sufficient to drive the conclusion that the two factors are anti-correlated. For example, tumor #5 has almost negligible levels of MTSS1 though it is not the sample with the highest miR-182 expression. Correlation coefficient should be calculated (and statistical significance provided) on a larger case series of specimens to prove anti-correlation. The same is valid for Fig. 5.

• Luciferase assay: it is not clear which of the two sites predicted to bind miR-182 has been mutated in the mut-3’UTR construct. Please clarify this point. Overall, the experiment should be described more in detail either in the text or in the figure legend.

• To demonstrate that miR-182 exerts its effect on invasion through MTSS1 (as reported in the Discussion “yet the ability of MTSS1 over-expression to
counteract miR-182’s pro-invasion effects…”), the authors should perform a rescue experiment, where miR-182 is cotransfected with a MTSS1 expression vector, mutated in miR-182 binding sites. In fact, if the effect on invasion mediated by miR-182 is ascribable to the suppression of MTSS1, ectopic expression of a mutant MTSS1, which is unresponsive to miR-182, is supposed to mitigate the effect of miR-182 on invasion. If this is not the case, it is likely that miR-182 exerts its effect through other mechanisms and the authors cannot exclude this hypothesis.

• Figure Legends are now provided as supplemental material but they must be moved to the main manuscript.

Minor essential revisions
• Table 1: what is meant with etiology?
• Fig. 2: labels B, C and D are not reported in the legend.
• Fig. 4: labels C, D are not indicated in the figure legend.
• Fig. 4 B,C,D. To validate the specificity of the results, I suggest to show the expression levels of the miRNA after transfection.
• Page 4: Change “expressing different endogenous level” to “expressing higher endogenous levels than HUH-1 cell line”.

Discretionary revisions
• The authors tested the expression of MTSS1 protein in HCC and adjacent normal tissues by IHC. It would be really informative to assess the expression of miR-182 by in situ hybridization on the same tissue sections.

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

Quality of written English: Needs some language corrections before being published

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