Reviewer's report

Title: MicroRNA-26b inhibits epithelial-mesenchymal transition in hepatocellular carcinoma by targeting USP9X

Version: 3 Date: 24 March 2014

Reviewer: Paolo Gandellini

Reviewer's report:

The manuscript appears largely ameliorated compared to the original submission. I would suggest the authors to include in the text also the experiments and figures provided to the referees (as main or supplementary text/figure), such as the measurement of mir-26b levels upon inhibition or overexpression (add to fig.2), luciferase assay with mutated 3'UTR (add to fig.4) etc...

Major compulsory revisions

1) My major concern is still the use of a unique control (NC) for oligonucleotides utilized for inhibition and overexpression of miR-26b. Actually, if it is clear that antisense oligonucleotide is single-stranded, it is not obvious that the molecule used for miRNA overexpression is single-stranded as well. Generally, double-stranded miRNA mimics or even precursors are used, for which a single stranded NC would not be appropriate. Please clarify this point and eventually repeat the experiments using the appropriate control for each oligo.

2) The other issue, already highlighted in my previous revision, is the calculation of p-values in fig.1. It does not make sense to calculate the p-value of the triplicate PCR for each sample compared to the control. Rather, it would be more informative, even if there are only 3 samples/group, to calculate the p-value of the difference between i) all tumors vs all normal tissues, ii) WHO I vs normal tissues and iii) each WHO vs the others. Eventually, iv) all cell lines together vs all normal tissues.

3) Show in the paper the measurement of USP9X mRNA levels upon miR-26b manipulation.

4) Figure 5: show levels of USP9X protein as reported in the figure 2 for the referee.

5) The sentence “the levels of cytokines...” in the discussion is still unclear. Please check English language.

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: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests