Reviewer's report

Title: MicroRNA-99a Induces G1-Phase Cell Cycle Arrest and Suppresses Tumorigenicity in Renal Cell Carcinoma

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Reviewer: Paolo Gandellini

Reviewer's report:

Cui et al. investigated the potential of miR-99a as a tumor suppressor miRNA in RCC and validated mTOR as a direct target of the miRNA. The study includes characterization of clinical samples and in vitro/in vivo investigations using cell cultures for manipulation of miR-99a expression. Overall, the findings are interesting and the manuscript well written.

Major compulsory revisions

• The use of a single-stranded molecule, such as that referred to as NC, as a control for siRNA-TOR is inappropriate. A scrambled double-stranded siRNA should be used instead. Please repeat the experiments reported in figure 7 using a control siRNA. In the same figure, miR-199a-3p has been reported as a label in the graph. Is it a typing mistake for mTOR-siRNA or did the authors report an experiment performed transfecting, miR-199a-3p?

Minor essential revisions

• It would be informative to give an estimate of the % of tumor or normal cells present in the clinical specimens used for expression studies (or at least indicate in the methods if a cut-off has been set to select the specimens to be used).

• Use the same y-scale in the upper plots of figure 3, to make the differences in the peaks more evident. The same is valid for figure 7C.

• Is mTOR expression inhibited in tumor xenografts after miR-99a injection?

• Minor corrections of English language are required.

Discretionary revisions

A phenocopy experiment has been performed to assess whether miR-99a phenotype can be ascribed to mTOR suppression. However, to specifically demonstrate the participation of mTOR to miR-99a phenotype a rescue experiment could be more appropriate. This means to verify whether miR-99a can induce its effects when mTOR is simultaneously exogenously expressed (or as an alternative by simultaneously inhibiting miR-99a and silencing mTOR). This experiment would more directly demonstrate if mTOR suppression is necessary to produce miR-99a phenotype.

Level of interest: An article of importance in its field

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