Author's response to reviews

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

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Author's response to reviews: see over
Dear editor: Enclosed is a manuscript entitled “MicroRNA-99a Induces G1-Phase Cell Cycle Arrest and Suppresses Tumorigenicity in Renal Cell Carcinoma” by Xiaozhou He et al., which we are submitting for publication in BMC Cancer.

In this study, our results demonstrate for the first time that miR-99a is frequently downregulated in renal cell carcinoma (RCC) tissues and correlates with overall survival of RCC patients. Moreover, deregulation of miR-99a is involved in the tumorigenesis of RCC partially via direct targeting mTOR pathway. These findings suggest that miR-99a may offer an attractive new target for diagnostic and therapeutic intervention in RCC.

Beside, we confirm that our manuscript has not been, or will not be submitted elsewhere for published, and all authors have read and approved the manuscript. We appreciate your consideration of our manuscript, and we look forward to receiving comments from the reviewers.

Yours sincerely
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Response to the reviewers’ comments and suggestion on MicroRNA-99a Induces G1-Phase Cell Cycle Arrest and Suppresses Tumorigenicity in Renal Cell Carcinoma submitted to BMC Cancer by Cui et al.

Reviewer: Paolo Gandellin

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**

1) 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. 2) Is it a typing mistake for mTOR-siRNA or did the authors report an experiment performed transfecting , miR-199a-3p?

**Response:** Thanks a lot for the valuable comments. 1) We agree with the reviewer’s opinion. When we performed the experiments of mTOR-knockdown, we really used the scrambled double-stranded siRNA as NC. In addition, in my experiments, the NC which we used was all double-stranded molecule. In materials and methods, we only mentioned the sequences of forward, Which did not mean that it was a single-stranded molecule. Now we have showed the sequences of reverse in our manuscript. 2) We apologized for this! It is really a typing mistake for mTOR-siRNA. This could be attributed to we still performing the experiments of miR-199a-3p at the same time.

**Minor essential revisions**

1) 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).
Response: All tissue samples (40 pairs) used in our study contained more than 80% tumor cells, which confirmed by pathological study post operatively. We have showed it in our manuscript.

2) 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.
Response: The upper plots of figure 3 and figure 7C is the raw data of flow cytometry. The Y-axis represents the number of the cells extracted by flow cytometry in each experiment. In order to ensure the accuracy of the results, the flow cytometry extracted cells are unlikely consistent in each test. In addition, the results of cell cycle expressed as a percentage, which does not require the flow cytometry extracted the same cells in each test. So we did not use the same y-scale in the upper plots of figure 3 and figure 7C, in order to ensure the authenticity of our study.

3) Is mTOR expression inhibited in tumor xenografts after miR-99a injection?
Response: Yes it is. We detected the expression of mTOR in tumor xenografts after miR-99a injection by Western blot, we found that intratumoral delivery of synthetic miR-99a induced a makedly inhibition of mTOR expression compared with control mice. The protein of tumor xenografts which we extracted previously has been stored in the refrigerator at -80 °C. We have showed it in our manuscript.

4) Minor corrections of English language are required.
Response: According to the reviewer's request, we modified the English language once again.

Reviewer: Annika Fendler
Reviewer's report:
The authors of the manuscript describe a tumorsupressive role of miR-99a in
renal cell carcinoma. In general the study is well-defined. The novelty of the result is nevertheless limited as miR-99a has been shown to be downregulated in several human cancers and to target the mTOR pathway before (see: Oneyama et al. Oncogene (2011) 30:3489–350. and Li et al. J Biol Chem. (2011) 286:36677-85.). Thus, the study only confirms this interaction in renal cell cancer. Some revision should be made to make the manuscript suitable for publication.

**Major compulsory revisions:**

The number of patients is rather small. To provide stronger evidence that miR-99a is downregulated in renal cell cancer, the data should at least be validated by bootstrapping or crossvalidation. Preferably, the authors should confirm their findings in a second sample set and provide appropriate statistics.

**Response:** Thanks a lot for the valuable comments. We agree with the reviewer's opinion. In our manuscript, we also mentioned that the relatively small number of clinical samples was a limitation to our study. According to reviewer's opinion, we confirmed our findings in a second sample set (another 20 pairs). In this way, we expanded the total number of clinical samples to 40 pairs, we found the expression of miR-99a was still remarkably downregulated in RCC tissues (29/40, 72.5%), compared with matched adjacent non-tumor tissues, which conformed our conclusion once again.