Author's response to reviews

Title: microRNA-141 inhibits cell proliferation and invasion and promotes apoptosis by targeting hepatocyte nuclear factor-3beta in hepatocellular carcinoma cells.

Authors:

Li Lin (yun.shally@163.com)
Hongwei Liang (lianghongwei0418@163.com)
Yanbo Wang (ybwang91@126.com)
Xiaomao Yin (907227716@qq.com)
Yanwei Hu (ywhu0618@163.com)
Jinlan Huang (504575084@qq.com)
Tingyu Ren (317073541@qq.com)
Hui Xu (604691406@qq.com)
Lei Zheng (nfyyzhenglei@smu.edu.cn)
Xi Chen (xichen@nju.edu.cn)

Version: 5
Date: 25 September 2014

Author's response to reviews:

September 20, 2014

Dear Editor,

Thank you very much for handling our manuscript entitled “microRNA-141 inhibits cell proliferation and invasion and promotes apoptosis by targeting hepatocyte nuclear factor-3beta in hepatocellular carcinoma cells” (Manuscript ID: 2145600751275455) submitted to BMC Cancer. We also greatly appreciated the constructive comments by the Reviewers.

Please see our point-to-point response letter and revised manuscript (the modified parts are underlined), we have vigorously revised our manuscript according to the reviewers’ comments. We feel that the revised manuscript is significantly improved.

Thank you very much for this matter and I am looking forward to hearing from you.

Sincerely,

Lei Zheng
Point-to-Point Response to Editor’s and Reviewers’ Comments:

Reviewer 1

1. I believe that Liu, et al. from the same hospital (Nanfang Hospital, Southern Medical University, Guangzhou) has reported the role of miR-141 in HCC. Please check the following publication: Liu, et al. MiR-141 suppresses the migration and invasion of HCC cells by targeting Tiam1. PLoS One. 2014 Feb 13;9(2):e88393. This must be included in the references and please explain that the data in the current study has never been reported elsewhere, including the publication of Liu, et al.

We greatly appreciate the reviewer for emphasizing this critical issue. We have cited and briefly discussed this paper in the revision. (Page 10, Line 235 to 237)

2. Only 8 pairs of HCC tissues were included in the current study. It is extremely a small number and the result is not convincing enough. The sample size needs to be expanded to confirm the founding.

We appreciate the reviewer’s emphasis on this critical issue. We have expanded the sample size in the revised manuscript. As shown in new Figure 1 and 2B, we have investigated the expression levels of HNF-3# and miR-141 in 4 additional pairs of HCC and noncancerous tissues. The results confirmed that HNF-3# protein levels were higher in HCC tissues compared with those in noncancerous tissues, whereas miR-141 levels were lower in HCC tissues. (Page 5, Line 100/ Page 6, Line 125 to 129/ Page 11, Line 261/ Page 20, Line 538, Line 541, Line 547 to 563)


We greatly thank the reviewer for this comment. We have cited and briefly discussed this paper in the revision. (Page 9, Line 212 to 213)

4. Please explain why miR-141 inhibitor was not transfected in the in vitro experiment? Otherwise please add these experiments.

We greatly thank the reviewer for this comment. Actually, we have tried to
transfect HepG2 cells with miR-141 inhibitor (anti-miR-141). However, because miR-141 was significantly downregulated in HCC tissues and cells, its levels were quite low in HepG2 cells. We could not observe significant inhibition of miR-141 in HepG2 cells after transfection with anti-miR-141. Therefore, we did not use miR-141 inhibitor in the in vitro experiment.

5. Please explain why only HepG2 cell line was used? Can one HCC cell line represent the general features of HCC population? It is better to repeat the experiments with other cell lines to provide sounder data.

We thank the reviewer for this excellent suggestion. As show in new Figure 2G-2I, we have confirmed the effect of miR-141 on HNF-3# in an additional HCC cell line, Huh7. (Page 6, Line 146 to 151/ Page 21, Line 558 to 563)

6. Liu, et al reported that MiR-141 suppresses the migration and invasion of HCC cells by targeting Tiam1. Some other targeting genes of miR-141 were also mentioned in the text: HDGF, MAP4K4 and GNA13. Here, Lin, et al. demonstrated that MiR-141 suppresses the viability and invasion of HCC cells by targeting HNF-3#. It is better to include the possible targets in the current study, especially Tiam1.

We thank the reviewer for this constructive suggestion. We totally agree with the reviewer that we should include the possible targets of miR-141 in this manuscript. We have discussed this in the revision (Page 10, Line 235 to 237).

7. The methods are appropriate and well described. However, if more assays could be performed for the functional tests (proliferation, invasion and apoptosis), the data will be stronger.

We understand the reviewer’s concern regarding this issue, and we totally agree with the reviewer that it is better to employ more assays to strengthen the data. Because the assays we used have been well established in measuring cell proliferation, invasion and apoptosis, we think it is no need to add more assays in the revised manuscript. We apologize for not following the suggestion of the reviewer and will conduct the regarding experiment in future.

8. Since miR-141 has been already reported to function as a tumor suppressor and inhibit the migration and invasion of HCC cells. The authors need to point out and emphasize the highlight of their own work, especially in the last paragraph of the paper.

We appreciate the reviewer’s constructive suggestion on this issue. We have emphasized the highlight of our own work in the last paragraph of the paper. (Page 10, Line 251 to 255)
9a. Too many errors were found in the English writing. For example: Line 1: promote # promotes.

We apologize for this error and have corrected it in the revision. (Page 1, Line 1)

9b. Line 43,44: This is not a complete sentence because the verb is missing.

We apologize for this error and have corrected it in the revision. (Page 2, Line 45 to 46)

9c. Line 58,59: Therefore, studying the molecular basis of HCC is vital for exploring new therapeutic agents# Better to be changed into: Therefore, it is vital to study the .......

We thank the reviewer for clarifying this issue for us and have modified it in the revision. (Page 3, Line 60 to 61)

9d. Line 68: Baroukh [8, 9] found: Baroukh is not the first author of reference 9, so the writing should be corrected. When there are more than one author in the references, please add “et al”.

We apologize for this error and have corrected it in the revision. (Page 3, Line 71)

9e. Line 75,76: Recent studies demonstrate that#Recent studies have demonstrated that...

We thank the reviewer for clarifying this issue for us and have modified it in the revision. (Page 3, Line 80 to 81)

9f. Line 82: Zhao’s team#Zhao, et al.

We apologize for this error and have corrected it in the revision. (Page 4, Line 87)

9g. Line 228: hepatic cells#HCC cells

We thank the reviewer for clarifying this issue for us and have modified it in the revision. (Page 10, Line 242 to 244)
9h. Line 230: miR-141-suppressed cell proliferation and invasion and miR-141-promoted apoptosis, # miR-141 suppressed cell proliferation and invasion and miR-141 promoted apoptosis,

We apologize for this error and have corrected it in the revision. (Page 10, Line 245 to 246)

9i. Line 254: 37# Should be corrected.

We apologize for this error and have corrected it in the revision. (Page 11, Line 274)

Line 294: each well was transfected#cells in each well were transfected

We apologize for this error and have corrected it in the revision. (Page 12, Line 314 to 315)

10. It is better to use “hepatocellular carcinoma” than “hepatic carcinoma”.

We thank the reviewer for clarifying this issue for us and have modified it in the revision. (Page 2, Line 45/ Page 4, Line 93, 95/ Page 11, Line 270)

11. The authors need to explain more in detail why HNF-3# were upregulated in some clinic samples and downregulate in the same samples.

We apologize for not indicating this issue clearly. In Figure 1A and 1B, we showed that the expression levels of the HNF-3# protein were uniformly upregulated in tumor samples. However, we found that the HNF-3# mRNA level appeared to be upregulated in some tumor samples and downregulated in other samples. The results suggest that a post-transcriptional mechanism is involved in the regulation of HNF-3#. We then experimentally validated that miR-141 directly regulated HNF-3# expression. (Page 6, Line 131 to 144)

12. What is the difference between “proliferation” and “viability”? Can a “Cell viability assay” (Line 328) detect cell proliferation (Line 1 in the title)?

We apologize for the confusion and have corrected it in the revision. The word “Cell viability assay” has been changed to “Cell proliferation assay”. (Page 14, Line 348, 349/ Page 21, Line 569, 571, 576)

Reviewer 2
1. The discussion is adequately supported by the data but it's not well balanced and requires a more in-depth and critical commentary of the results.

We thank the reviewer for clarifying this issue for us. We have re-written the Discussion section to add in-depth and critical commentary of the results. (Page 9 to Page 10)