Author’s response to reviews

Title: MicroRNA-21 promotes hepatocellular carcinoma HepG2 cell proliferation through repression of mitogen-activated protein kinase-kinase 3

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Author’s response to reviews: see over
Re: MS: 8009238869951921R1

Response to Reviewer Comments

Dear Dr. Mazzocca,

Please find enclosed a revision of our manuscript entitled "MicroRNA-21 promotes hepatocellular carcinoma HepG2 cell proliferation through repression of mitogen-activated protein kinase-kinase 3". (Manuscript ID 8009238869951921). We thank you and the reviewers for the thoughtful comments and productive suggestions. We have improved our manuscript by addressing the reviewers’ concerns. Attached is a point-by-point outline of changes made in response to the comments, and we have also marked the changes in the text with colored font.

Thank you for handling our manuscript, we are looking for hearing from you in future.

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Reviewer #1:

We thank the reviewer for his/her positive comments and constructive suggestions on our manuscript. Below we have addressed each of the reviewer’s concerns (C) followed by our response (R).

Comments (C):
Minor Essential Revisions

C1: please add "not" after has in line 7 of paragraph 3 from introduction.
R1: Thanks. We have made the change.

C2. change "A" to C in row 6 on figure 1 legend.
R2: We corrected the error, thanks.

C3. should add statistic analysis result on figure B in Figure 2.
R3: We marked the statistical significance in the figure accordingly. Thanks.
C4. should add the results of Ad/MiR-21 control on Figure 5.

R4: Thanks for the helpful comment. Since a large body of studies has verified the miR-21 is able to promote cancer cell proliferation including the HCC HepG2 cells (Zhu et al., 2012), we didn’t perform this control.

Reviewer #2:
We thank the reviewer for his/her helpful suggestions on our manuscript. Below we have addressed each of the reviewer’s concerns (C) followed by our response (R).

General comment:
In this manuscript, the authors have identified MAP2K3 as a novel target of oncomiR, miR-21. Besides the importance of miR-21 in cancer and the impact of their novel target gene, some indispensable data was missing. Several points need clarifying.

Response: We thank you for your constructive comments and the time and efforts you have put on our MS.

Major concerns (C)

C1. The authors described the repression of MAP2K3 was inversely correlated with miR-21 (in the Abstract, page 2 line 7.). Although, there are no results of miR-21 expression status using the corresponding tumor and non-tumor samples. While it is well known that miR-21 is overexpressed in tumor samples, the conclusion that these are correlated is unclear. In addition, the authors used qRT-PCR for miRNA expression analysis. How does the miRNA with only 22nt small RNA could be amplified with the primer? The authors should mentioned the reference or show detailed methods if they don’t use widely used materials such as TaqMan probe.

R1: Thank you for raising the critical concerns. In the present study, we found the expression of MAP2K3 was dramatically down-regulated in HCC tissues relative to their matched non-tumor controls as determined by immunohistochemical staining assay. Together with the previous findings by Ladeiro et al. that miR-21 was elevated in malignant HCC tissue relative to the benign HCC and normal liver tissues (Ladeiro et al., 2008), and by Jia et al. that MAP2K3 was down-regulated in breast cancer epithelial cells (Jia et al., 2010), we therefore hypothesized that miR-21 might has a correlation with MAP2K3 in HCC. We have incorporated the rational into the introduction part of revised text. In addition, we technically employed qRT-PCR to quantify miR-21, in which a primer containing the loop sequence able to specifically recognize matured miR-21 was used for first-strand of miR-21 synthesis by reverse transcription. The qRT-PCR assay has been used in miRNA profile analysis with high fidelity sensitivity and reproducibility (Chen et al., 2009; Balcells et al., 2011). These references were provided in the revised text accordingly.
C2. The authors used only HepG2 cells for the entire experiments as they presented in the title. It is not sure that using HepG2 cell for overexpression experiment is appropriate and reasonable while HepG2 are previously reported to overexpress miR-21 compared with normal liver tissues (Zhu et al., Oncol Rep. 2012). The authors also should mention the expression status of HepG2 in the main text by showing data or adding some references.

R2. This is really a great concern. We clarified and stated the endogenous miR-21 expression with the reference in the revised text. Thank you.

C3. Some of the control experiments were missing in the Luciferase reporter assay in Fig.2B and C. First of all, the authors should add the results of co-transfection of pMIR-Report empty vector with pAd/con or pAd/pri-miR-21 for Fig.2B and pAd/con or pAd/miR-21/inhibitor for Fig.2C to exclude the possibility of off-target effect. In addition, the asterisk should show which is the pair to be compared.

R3. We agreed with the reviewer’s comment on the control experiment and it would be better if these controls were included. In the study of dual-luciferase reporter assay, a pMIR-report alone was served as control for pMIR-report and transfection efficiency. Since theoretically, however, there was no interaction between pAd/con or pAd/miR-21/inhibitor, we did not set the combination of pMIR-report and pAd/con or pAd/miR-21/inhibitor as controls to rule out the off-target effect might caused. Thank you.

C4. The sentence on page 12 line 8, describe the repression of MAP2K3 in HCC is a evidence to show MAP2K3 as a target of miR-21 is misreading. The authors should describe why they focused on MAP2K3 while they are plenty of predicted targets for miR-21.

R4. We agreed with you, it was overstated here at this point. We have deleted this statement, and instead of insertion of a sentence “Since miR-21 has been demonstrated to be elevated in many types of cancer, including the HCC,” at the beginning of follow paragraph for language transition in the revised text. Thank you. As addressed in concern #1 (C1), we did add the rational of why the MAP2K3 was chosen as target of miR-21 in this study. Mainly since the evidence of miR-21 was elevated in HCC and MAP2K3 was down-regulated in breast cancer cells (Jia et al., 2010), led us focus the MAP2K3 as target of miR-21.

References:

Balcells, I., S. Cirera and P.K. Busk, 2011. Specific and sensitive quantitative rt-pcr of mirnas with DNA primers. BMC biotechnology, 11: 70. Available from 

