Author's response to reviews

Title: Special Role of Foxp3 for the Specifically Altered microRNAs in HCC-activated Regulatory T Cells

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Author's response to reviews: see over
Cover letter

Dear Editor,

We would like to submit the enclosed manuscript entitled "Special Role of Foxp3 for the Specifically Altered microRNAs in T regulatory cells of HCC patients" for your consideration for publication in *BMC Cancer* as a research article. This modified manuscript is a revised version of our previous one (MS: 1461900899121743).

We are most grateful to you and the reviewers for your very helpful suggestions and comments related to our previous manuscript. We have carefully read the comments and rewritten the manuscript. To improve the style of written English, one of our co-operators in USA has copyedited the manuscript before being revised. Our point-to-point responses to the comments are attached in the following response to reviewers.

The manuscript is NOT under consideration for publication elsewhere. We also confirm all the authors have approved the submitted manuscript. The authors do not have any possible conflicts of interest.

Thanks so much for your consideration!

Sincerely yours,

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Response to Reviewers

Reviewer's report:

…In this manuscript the authors identified 9 specifically altered miRNAs in HCC-activated Tregs …

…The novelty of the study is high and the authors describe the first evidences that Tregs in HCC exhibit specifically altered expression of some miRNAs, …

…These results are useful both in finding new biomarkers and in further exploring the functions of Tregs in HCC patients. However, to accept this article some corrections and clarifications are needed. ..

Minor Essential Revisions

1. In the part of the study focusing clinical samples, authors compared miRNA profiles in Tregs of HCC patients and controls. There is now direct evidence of HCC-specific activation of Tregs in HCC patients in the study, title should be corrected accordingly (e.g. Special Role of Foxp3 for the Specifically Altered microRNAs in T regulatory cells of HCC patients)

Thanks so much for the good suggestion. Following your suggestion, we have changed the title from “Special Role of Foxp3 for the Specifically Altered microRNAs in HCC-activated Regulatory T Cells ” to “Special Role of Foxp3 for the Specifically Altered microRNAs in T regulatory cells of HCC patients” in the revised manuscript. (Please refer to the Title Part, Page 1 Line 1-2)

2. Please explain the reason for choosing miR-344e-5p as negative control (page 10)? What are the arguments in favor of this miR in this experimental context?

The expression level of miRNAs was normalized by the endogenous U6 level with the comparative Ct method (ΔCt method), and we chose the miR-344e-5p as the negative control to further verify the credibility of validation. Because this miRNA did not pass volcano plot filtering (fold change = 1.85, P-value = 0.54) in microarray, therefore, we chose it as the negative control to verify the credibility of our qRT-PCR process; we also gave a brief explanation in the revised manuscript. (Please refer to the Result Part, Page 8 Line 13-15 )
3. You claimed that you used specific primers for mRNA transcription to cDNA (page 13). Which ones? Please clarify.

Thanks a lot for your suggestion. We have clarified all the related primers by two tables the revised manuscript. (Please refer to Supplementary Table 1 and Supplementary Table 2)

4. The abbreviation for Fluorescence-activated cell sorting should be FACS and not FACs. Please make the change.

Thank you for your careful reading. We have changed the abbreviation.

5. Figure 1: Please increase the space between figure A and C to avoid confusion

Thank you for your kindly reminding. We have increased the space of figure A and C in Figure 1. (Please refer to Figure 1).