October 16, 2021

Dear Professor Ma,

Enclosed please find our revised manuscript (NO: 71435) entitled "The dynamics of cytokines predicts the risk of hepatocellular carcinoma among chronic hepatitis C patients after viral eradication". As suggested by the reviewers, we had revised the manuscript and all the modifications were highlighted in yellow. Our point-by-point replies to the reviewers were as follows:

Reviewer #1
1. The abstract section can improve—add a focus point in the abstract section.
Response: We had revised the section of abstract.

2. Rewrite the conclusion (in the abstract) in a more straightforward form.
Response: The conclusion (in the abstract) had been revised as "Downregulation of serum TNF-α significantly increases the risk of HCC after HCV eradication. A predictive model consisting of cytokine kinetics could ameliorate personalized HCC surveillance strategies for post-SVR HCV patients."

3. The primary endpoint was the development of *de novo* HCC. What does it mean?
Response: *de novo* HCC means new-onset hepatocellular carcinoma.

4. Is it necessary to show the HCC predictive model in the abstract?
Response: HCC predictive model had been deleted in the abstract.

5. Authors are suggested to use the full form when used for the first time throughout the manuscript.
Response: All the abbreviations had been defined upon the first appearance in the manuscript.

6. The introduction section looks good. Authors can try to include the existing

research limitations also, how the present research unravels those limits.
Response: There are some unsolved issues regarding new-onset HCC after SVR. The identification of the HCV patients who maintain a high risk of HCC following successful antiviral therapy remains an unmet need. The mechanism of hepatocarcinogenesis despite HCV clearance is still unclear.

7. Aim of the study should need to add as the last paragraph in the introduction.
Response: Aim of the study had been added to the last paragraph in the introduction. First, this study aimed to investigate the impact of differential cytokine expression profiles on the development of HCC among chronic hepatitis C patients with advanced fibrosis who achieved SVR. Second, we attempted to identify immune biomarkers to predict the risk of HCC after successful antiviral therapy.

8. Material and methods also look good. Need a logical flow of the writings with enough references.
Response: We had strengthened the section of material and methods. We had added the references regarding the rationale of SVR 12 for the DAA group and SVR24 for the IFN group (Ann Hepatol 2016; 15(2): 154-159; PLoS One. 2021 Feb 4;16(2):e0245479). The method of multiplex immunoassay had been briefly described in the paragraph of cytokine measurement.

9. Check all the gene symbols.
Response: We had carefully checked all the gene symbols (supplementary table 3).

10. Sustained virologic response was defined as undetectable HCV RNA throughout 12 weeks (SVR12) for the DAA group or 24 weeks for the IFN group (SVR24) after completion of antiviral therapy. Any references?
Response: SVR24 was considered the gold standard to define the successful eradication of HCV. SVR12 could reliably predict SVR24 in several populations infected with HCV (treatment-naïve, prior null responders, different genotypes) using various new DAA regimens. (Ann Hepatol. 2016;15(2):154-9.; PLoS One. 2021 Feb 4;16(2):e0245479.)

11. This assay was performed according to the manufacturer's instructions. Need a brief description.

Response: The method of multiplex immunoassay had been described in the paragraph of cytokine measurement. In brief, a calibration curve based on 1:3 dilutions of the highest standard was used for quantification. Beads were premixed and put into wells containing diluted serum and reagents. After fixation of the antigen on the capture antibody linked to the microsphere, a biotinylated detection antibody was added. The concentration of the analyte was quantified based on the bead color and the intensity of the fluorescent signal using the multiplex Luminex-200 (Luminex Corporation, Austin, TX). All samples were analyzed in duplicate.

12. The results section can improve by adding significant results.
Response: The significant results had been summarized in the first paragraph of the discussion.

13. The writing of results is good. Need to maintain a logical flow of the writings.
Response: We had consulted a native English-speaking expert to improve the logical flow of the writings.

14. Figures presentation is up to mark.
Response: All the figures had been organized into a single PowerPoint file (71435-Figures.pptx).

15. Figure legends are not self-explanatory. Need to confirm without the repetition of the results and discussion in the figure legends.
Response: We had deleted the repetition of the results and discussion in the figure legends.

16. The discussion is good. The discussion section can improve by including the data from other sources about related works.
Response: We had strengthened the literature review in the discussion.

17. The conclusion needs to address future perspectives.
Response: We had revised the conclusion. Our findings provide a clue for the pathogenesis of hepatocarcinogenesis and a strategy for HCC surveillance based on risk stratification. With the development of high-throughput molecular technology, it is believed that more novel biomarkers will be applied in the early detection of HCC in the future.

18. Novelty of the work should be added by the author in the conclusion section.
Response: We had revised the conclusion.

19. Many spacing, punctuation marks problems found in the tables.
Response: We had corrected the errors of spacing and punctuation marks in the tables.

20. Spacing, punctuation marks, grammar, and spelling errors should be reviewed thoroughly. I found so many typos throughout the manuscript.
Response: The revised manuscript had been sent to an English language editing company to polish the manuscript further and verified by a new language certificate.

Reviewer #2
I don't find any challenges in the manuscript, but some sentences could be better formulated. The graphics need to improve.
Response: The revised manuscript had been sent to an English language editing company to polish the manuscript further and verified by a new language certificate. We had improved the graphics according to the guidelines for manuscript revision. All the abbreviations in the graphics had been defined in the footnote. All the figures were decomposable and had been organized into a single PowerPoint file (71435-Figures.pptx).

Sincerely Yours,

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