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20 November 2022

Dear Editor and Reviewer,

Please find enclosed the revised version of our manuscript entitled "Transcriptome Analysis Creates a New Era of Precision Medicine for Managing Recurrent Hepatocellular Carcinoma".

Thank you for reviewing our manuscript. We feel that all the comments are very helpful in improving the legibility, objectivity, and scientific evaluation of the manuscript.

In the revised manuscript, in accordance with the valuable suggestions of the reviewers, we have made various modifications. All the revised parts are highlighted in red with Microsoft Word "Track Changes" function. The answers made are detailed, point-by-point, in a letter to the reviewer.

We hope that the revised manuscript will retain your attention, and that you will judge the revised manuscript to be suitable for publication in *WJG*.

Thank you.

Yours truly,
Wey-Ran Lin

Reviewer #1:

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Specific Comments to Authors: This review details how various transcriptome profiling methods have contributed to our understanding of recurrent HCC with respect to the carcinogenicity of primary cancer cells, carcinogenic stimuli, and tumor microenvironments. The results are true and authentic. The author scientifically and reasonably cite the latest references which are important in this field and related to the scientific problems and research hypotheses addressed in the study. The language in article correctly, clearly and concisely express the information.

Response: Thank you for your impressive comments. We hope this article can interest more physicians and scientists involved in this field and facilitate collaborations. Eventually, we can benefit cancer patients and satisfy unmet needs in the real world with state-of-the-art technologies.

Reviewer #2:

Specific Comments to Authors: In this manuscript, the authors summarized some findings yielded with these transcriptome methods of for managing recurrent hepatocellular carcinoma. However, there're some issues which should be addressed. 1. Most of the contents and conclusions have been reported. Please explain the novelty of this manuscript in detail. 2. Some of the content is basic knowledge and should be deleted or reduced substantially, especially the introductory and technical content on PCR, Micaoarray, and RNA-Seq Analysis. Quantitative Reverse Transcription Polymerase Chain Reaction Analysis of Recurrent HCC; 3. Important references (authors, research content, results, important research methods, etc.) should be listed in some tables. Major revision should be recommended.

Response: Thank you for your insightful comments. We have addressed the points that you raised below:

1. Regarding the novelty, we notice that the principles of how transcriptomic techniques work as well as some of the cited studies in our article were summarized by other peers previously. However, we believe that several elements contribute to the novelty in our review compared to previous work. First, previous work mainly focused on methodologies (the principles and the extended methods of each technique, the study designs, and the aims of studies) but to a lesser degree reflected on the results yielded by all these research tools. As mentioned on page 4, line 96-99 and Figure 1, we believe one

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of the strengths of our article is to organize the findings of a wide variety from the clinicopathological view and try to endow them with some plausibility that is worthy of further investigation and validation. Second, molecular diagnostics has advanced rapidly over the past two decades, especially in the era after the breakthrough of next-generation sequencing. We not only provide a comprehensive review to introduce the development and the progress of available transcriptome analyzing tools in chronological order but also compare the techniques under the HCC-specific context, and emphasize the yields and some issues arising from practices over the years (page 5-6, line 129-138) that have not been thoroughly reviewed yet. Third, even though transcriptome analyses in HCC and related literature reviews are common, studies intending to look at HCC recurrence are relatively limited, and we try to list the available research on this topic, including the most updated scRNA-seq data published in the second half of 2022. Lastly, we added a paragraph in the revised manuscript (page 21-22, line 537-564) that highlights the gap between the bench and bedside and denotes the importance of translating these techniques to the clinical field. We hope this review help draw the attention of clinical practitioners and call on members of health authorities to facilitate the applications of these technologies.

2. Thank you for your comment. The revision has been made accordingly. We deleted the technical contents (page 5, "QUANTITATIVE REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION ANALYSIS OF RECURRENT HCC" first paragraph; page 7, "MICROARRAY ANALYSIS OF RECURRENT HCC" first paragraph; page 13, "RNA-SEQ ANALYSIS OF RECURRENT HCC" second paragraph) of the three transcriptomic approaches in the text but preserved the basic steps in table 1 for those who would like to refresh on how these techniques work.

3. Thank you for your valuable suggestion. We took your advice and cataloged the representative studies on this topic with information including authors, references, methods, and major findings specified in the new Table 2. We also added corresponding citations for "Applications and main achievements in HCC recurrence-related research" in the revised Table 1.

Reviewer #3:

Specific Comments to Authors: This is a very interesting, updated, and well-written manuscript highlighting a crucial point in cancer diagnosis. In spite of the huge advance

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in OMICs techniques and particularly in transcriptomics current diagnostic and monitoring guidelines in HCC are still based on conventional methods such as AFP quantitation and imaging assessments. But I think that this manuscript brings another message; it is time to move toward the next generation of diagnostic tools in modern medicine, most of them are available, and validated. In this regard, the only point I recommend is to add a new paragraph just denoting that now the limitation is not the technology, the main limitation is to count with proper health policies and a critical mass of physicians who know the scope, and the relevance of Omics techniques, which can provide today a direct benefit for patients in many diseases including cancer.

Response: Thank you for pointing out this important issue. We have added a new paragraph “Limitations and Future Directions” of omics technologies at the end of the manuscript, which covers the current obstacles, the health policies, and the multidisciplinary networks that need to be improved nowadays. Please refer to page 21-22, line 537-564 of the revised article.