Response Letter

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World Journal of Clinical Oncology

Dear editors and reviewers:
Thank you for your letter and for reviewers' comments concerning our manuscript entitled "Identification of the Key Genes and Mechanisms Associated with Transcatheter Arterial Chemoembolisation Refractoriness in Hepatocellular Carcinoma (Manuscript ID: 89149). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our research. We have studied comments carefully and have made correction which we hope to meet with approval.

Reviewer #1:

Specific Comments to Authors: Dear Authors, Thank you for submitting your work to the World Journal of Clinical Oncology. This is a very interesting and of high quality article on the identification of genes that might predict response to TACE. The Introduction section is informative and provides adequate background. Methodology is sound, although I would like to know why you selected only 5 genes for your further analysis. Results are presented in thorough detail with appropriate tabulations and figures. Discussion is comprehensive. However, a paragraph should be included discussing the limitations of the study. For instance, the heterogeneity in diagnosis, and treatment selection of all cases registered in accessed Databases, might have an impact on your results. Moreover, it would be important to know if there are data regarding previous treatment (prior to TACE) for these patients, as well as whether according to current guidelines, these patients should have been offered some other first-line treatment.

Q1: Methodology is sound, although I would like to know why you selected only 5 genes for your further analysis.

Authors' Response: Thank you for your questions. In this study, TACE refractoriness-related genes obtained by TACE-associated differentially expressed genes (DEGs), up-regulated HCC-associated DEGs and the genes in the TACE non-response trait significant modules were used to analyze via MCC algorithm in Cytoscape software (Version 3.7.2) for identifying the key genes. Cytoscape (version 3.7.2) is an open-source bioinformatics software platform for visualizing molecular interaction networks and MCC algorithm as a scoring method has a better performance on the precision of predicting essential proteins from the yeast PPI network than other 11 algorithms within Cytoscape's plug-in cytoHubba. According to literature review, the MCC algorithm was used to extract the top 20 branch marker genes (key genes) in the network. A protein with higher scores represents indicates it is associated with more proteins in the network. According to scoring results of TACE

refractoriness-related genes, only proteins of the five genes (DLGAP5, KIF20A, ASPM, KIF11 and TPX2) had relative high scores, while the rest had extremely low scores. To ensure the high reliability, we only selected the five genes as key genes associated with TACE refractoriness. Concerned about this question, we have made elaboration in page 8 line 29, page 9 line 1-5 and page 13 line 13-16.

Q2: Discussion is comprehensive. However, a paragraph should be included discussing the limitations of the study. For instance, the heterogeneity in diagnosis, and treatment selection of all cases registered in accessed Databases, might have an impact on your results.

Authors' Response: We are very sorry for our negligence of discussing the limitations. We have supplemented the limitations of this study in discussion part. After carefully considering, the limitations of this study are in page 19 line 5-16:
"However, some limitations exist in this study. Firstly, although the five key genes (DLGAP5, KIF20A, ASPM, KIF11 and TPX2) were verified as being associated with TACE refractoriness, the clinical applicability of these genes requires more cases as confirmation due to the limited number of TACE-treated patients in this study and the heterogeneity in the diagnosis. Secondly, due to insufficient samples of HCC cells and stroma treated by TACE, we have no scope for the further validation of the five key genes. Thirdly, the results of the AUCell analysis that TACE refractoriness-related genes were mainly active in hepatocytes and embryonic stem cells require further verification due to only one cell reaching the AUC threshold of TACE refractoriness-related genes list. Lastly, the potential mechanisms identified also need further proof through additional vivo and vitro experiments."

Q3: Moreover, it would be important to know if there are data regarding previous treatment (prior to TACE) for these patients, as well as whether according to current guidelines, these patients should have been offered some other first-line treatment.

Authors' Response: This question is definitely worth considering and we have confirmed that the inclusion criteria for patients contains histological confirmed HCC and with no previous treatment by consulting the registered clinical trial (https://clinicaltrials.gov/study/NCT00493402). Therefore, the enrolled patients did not accept any treatment before taking biopsy for histological diagnosis and treating TACE for study. For this question, we have made specific illustrations in page 6 line 29 and page 7 line 1-3:
"The datasets included were required to belong to *Homo sapiens* and contained HCC tissues from TACE non-responders and TACE responders who had not received any prior treatment."

Thank you for your kind reminders and consideration again.

Best regards,
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