September 20, 2016

Dear Editor,

Please find enclosed the edited manuscript in Word format
(file name: 29526 -Revised manuscript.doc).

Title:An update of genetic alterations in hepatocellular carcinoma

Author: Zhao-Shan Niu, Xiao-Jun Niu, Wen-Hong Wang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 29526

The manuscript has been improved according to the suggestions from you and the Reviewers:

1. The format has been updated

- Copyright agreement was uploaded
- A copy of signed Conflict-of-interest statement was uploaded
- Audio core tip was uploaded
- Language certificate was uploaded

2. Revisions have been made according to the suggestions of the Reviewers.**The changes in the revised manuscript are highlighted in red.**

⑴Reviewer: 02860809

The manuscript entitled "An update of genetic alterations in hepatocellular carcinoma" by Zhao-Shan Niu, Xiao-Jun Niu, Wen-Hong Wang is a very exhaustive and deep review on what is known about genetic alteration in the initiation, progression, maintenance of HCC with many comments on the possible implication on diagnosis prognosis and other clinical parameters. It is a complete and a very well written review that it is almost ready for

publication.

Response:Thank you for your positive comments.

①Are there any reports that establish an association between micronucleus on prognosis or overall survival in HCC? Any evidence published that allows to propose micronucleus as a diagnosis tool in HCC?

Response: So far, no reports have established an association between micronucleus and prognoses or overall survival in HCC. In addition, micronucleus scores in HCC have not been used as a diagnosis tool in HCC. Only one published report has found that micronucleus scoring has apotential use as an ancillary tool for diagnosing HCC using fine needle aspiration (FNA) cytology[1]. In our opinion, however, it is of minimal practical clinical significance. Therefore, we did not include these aspects related to micronuclei in this article.

1.**Wen CH,** Lin CH, Tsao SC, Su YC, Tsai MH, Chai CY.Micronucleus scoring in liver fine needle aspiration cytology.*Cytopathology*2013; 24:391-395 [PMID: 22974178 DOI: 10.1111/cyt.12009]

②Giving the fact that variability/inconsistency of the SNP GWAS studies, would authors recommend effort in this direction? Would recommend to keep studying SNPs in HCC as a marker of initiation and progression in HCC?

Response: The final paragraph in the SINGLE-NUCLEOTIDE POLYMORPHISMS section recommends efforts in this direction to investigators. ---"Taken together, the available results show that most findings related to the SNPs detected in GWAS on HCC can be problematic …Therefore, further well-designed investigations with larger sample sizes and multiple races/ethnicities are warranted to elucidate the impact of SNPs on the susceptibilty to HCC."

Most SNPs detected in GWAS on HCC can be problematic to replicate due to

differences in different racial/ethnic groups, different study designs, and genetic heterogeneity. The following content has been added to the third paragraph in the **SINGLE-NUCLEOTIDE POLYMORPHISMS section** in the revised manuscript.

"Given the high variability/inconsistency in findings related to SNPs found in GWAS, at least to date, we cannot recommend the continued study of SNPs in relation to HCC as a means for identifying reliable markers of the initiation and progression of HCC."

③Are there any reports assessing TP53 mutation status in HCV infected patients?

Response: There are a few reports assessing *TP53* mutation status in HCV-infected patients. We have added text, i.e., "with similar results found between patients with HBV infections and HCV infections", based on Ref. 139 and 140, to the fifth sentence of the fifth paragraph in the ***TP53*** section in the revised manuscript, which now reads as follows.

"In addition, two systematic reviews concluded that *TP53* mutations were associated with poor OS, relapse-free survival rates (RFS), and DFS in HCC patients, with similar results found between patients with HBV infections and HCV infections[139,140]."

④Would authors propose hTERT mRNA levels measurement as a diagnostic tool in HCC?

Response:We propose the measurement of *hTERT mRNA* levels as a diagnostic tool for HCC. As suggested, the following content has been added to the main body as the sixth paragraph in the ***Telomerase reverse-transcriptase section*** in the revised manuscript.

"*hTERT* mRNA has been reported to be detectable in the serum of patients with HCC, andit has been reported that the sensitivity and specificity for serum *hTERT* mRNA in detecting HCC are 77.14% and 100%, respectively, which are higher than the sensitivity and specificity for AFP in the early

detection of HCC[171]. In another report, the sensitivity/specificity for serum *hTERT*mRNA in diagnosing HCC was found to be 90.2%/85.4%, which is superior to using alpha-fetoprotein (*AFP*), *AFP-L3*, and des-gamma-carboxy prothrombin (*DCP*) in the diagnosis of HCC at an early stage[172]. Therefore, measuring serum *hTERT* mRNA levels might serve as a potential diagnostic tool for HCC."

171. **El-Mazny A,** Sayed M, Sharaf S. Human telomerase reverse transcriptase messenger RNA (TERT mRNA) as a tumour marker for early detection of hepatocellular carcinoma. *Arab J Gastroenterol* 2014; **15**:68-71 [PMID: 25097049 DOI: 10.1016/j.ajg.2014.04.001]

172.**Miura N,** Osaki Y, Nagashima M, Kohno M, Yorozu K, Shomori K, Kanbe T, Oyama K, Kishimoto Y, Maruyama S, Noma E, Horie Y, Kudo M, Sakaguchi S, Hirooka Y, Ito H, Kawasaki H, Hasegawa J, Shiota G. A novel biomarker TERTmRNA is applicable for early detection of hepatoma. *BMC Gastroenterol*2010; **10**:46 [PMID: 20482774 DOI: 10.1186/1471-230X-10-46]

⑤I miss data on TGF-beta pathway.

Response:In HCC tissues, the overexpression of TGF-βwas found to be correlated with the carcinogenesis, progression, and prognosis of HCC[1]. However, TGF-β mutations have not yet beendocumented in HCCpatients[2]. Abnormalities of the TGF-β pathwayaxis are most often observed in HCCs that are poorly differentiated[3] and at an advanced stage[2]. Therefore, the TGF-β pathway may be explored as a therapeutic target for treating advanced HCC. In fact, the role of the TGF-beta pathway in the initiation and progression of HCC remainsunclear; thus,we did not include a discussion of the TGF-beta pathway in this article.

1. **Malaguarnera G**, Giordano M, Paladina I, Berretta M, Cappellani A, Malaguarnera M. Serum markers of hepatocellular carcinoma. *Dig Dis Sci* 2010; **55**:2744-2755 [PMID:20339916 DOI: 10.1007/s10620-010-1184-7]

2. **Neuzillet C,** de Gramont A, Tijeras-Raballand A, de Mestier L, Cros J, Faivre S, Raymond E. Perspectives of TGF-betainhibition in pancreatic and hepatocellular carcinomas.*Oncotarget*2014; **5**:78-94 [PMID: 24393789]

3. F**uruta K,** Misao S, Takahashi K, Tagaya T, Fukuzawa Y, Ishikawa T, Yoshioka K, Kakumu S. Gene mutation oftransforming growth factor beta1 type II receptor in hepatocellularcarcinoma. *Int J Cancer* 1999; **81**:851-853 [PMID:10362128]

⑥Section "problems and perspectives" accumulates some grammar mistakes: "there exist…"; "be widely realized…"

Response: We have changed "there exist…"to"there are…"in the revised manuscript. In addition, we have changed the statement "it has be widely realized…" to "it has been widely realized…" in the revised manuscript.

⑦Abbreviation list is extremely short. There are many abbreviations missing.

Response: We have added missing abbreviations in the revised manuscript.

⑵Reviewer:02936743

The article entitled"An update of genetic alterations in hepatocellular carcinoma" provides a comprehensive and insightful overview of genetic changes in hepatocancerogenesis. The authors make a systematic contribution to the research literature in this area of investigation. They documented different genetic alterations connected with hepatocancerogenesis and summarized them on Figure 1 and in three Tables. The presented information are relevant and theory based. The paper is ready for publication however needs minor revision.

Response:Thank you for your positive comments.

①The authors should revise the style and language used in the article. Using very long sentences should be avoided as they make the content difficult to follow.

Response:This manuscript has been re-edited by American Journal Experts (AJE)[Certificate Verification Key: 9E91-239D-88EE-8F23-0274].

②There are also some sentences used for the second time, e.g. "these findings suggest that beta-catenin mutations might need to cooperate with other oncogenic alterations or pathways…" and three lines below the same sentence: "the findings of the studies indicate …."

Response:Considering the Reviewer's suggestion, we have deleted the sentence "These findings suggest that β-catenin mutations might need to cooperate with other oncogenic alterations or pathways to result in hepatocarcinogenesis."from the revised manuscript. We are sorry for our repeated descriptions. Meanwhile, we have very carefully re-checked the entire manuscript to avoid repeated descriptions.

③There are many citations of the literature data used only once and provides sometimes only very general information, e.g. in subchapter of Wnt/beta-catenin signaling pathway. I feel that some citations are improper used. Please check for example "deregulation of WNT/catenin signaling was found in 40-70% of HCC patients" and citation no 234, or citation numbers: 244, e.g. pos. 248 – concerns not HBV, but HCV-induced HCC, 290 – is a mouse model of study, etc.

Response: Spot on! Thank you for your close attention!

We have carefully checked the references cited, and we found that "deregulation of WNT/catenin signaling was found in 40-70% of HCC patients"citesreference 234.

"In HBV-related HCC, *β-catenin* mutations have been found at a lower frequency"citesreferences 103, 244, and245 (103,246, and 247 in the revised manuscript.

We have deleted reference 248 from the revised manuscript.

Reference 291 has been used instead of reference 290, and reference 290 has been deleted from the revised manuscript.

The following reference has been used instead of reference 291 in the revised manuscript.

292. **Yu L,** Zhang J, Guo X, Li Z, Zhang P.MicroRNA-224 upregulation and AKT activation synergistically predict poor prognosis in patients with hepatocellular carcinoma.*Cancer Epidemiol* 2014; 38:408-413 [PMID: 24923856 DOI: 10.1016/j.canep.2014.05.001]

In addition, references cited have been checked carefully for accuracyin the entire manuscript.

④In gene and protein nomenclature please use italic for a symbol when the gene is meant and plan (roman) for when the proteins is meant.

Response: We have written gene initalics and protein namesinplan (roman)as appropriate in the revised manuscript according to the Reviewer's suggestion.

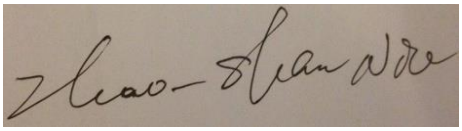
⑤I would also like to see the authors improve Figure 1 (more details regarding the title of the manuscript, colours, novelties) to be more professional and perfect.

Response:We have improved Figure 1in the revised manuscript according to the Reviewer's suggestion.

3. References and typesetting were corrected.

Thank you again for publishing our manuscript in the World Journal of Gastroenterology.

Sincerely yours,



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