Response to the reviewer and editor

Dear Editor:

Thank you for your positive response.

Below is our response to the comments.

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 23878

Title: Liver cancer stem cell markers: Progression and therapeutic implications

Authors: Jinghui Sun; Qing Luo; Lingling Liu; Guanbin Song

To Reviewer #1

Reviewer's code: 00053659

Comments to the author:

1. ***In the section 2, the marker list does not consistent with the order of the Table.***

Response: Thanks for your comments. On the basis of your suggestion, we have made the corresponding correction in the table based on the order of markers listed in the article. The markers listed in the table are consistent with the order in section 2 (Page 34, Table 1, red ink).

2. ***In the section 3, additional figure should be presented for understanding the hierarchy of the cancer stem cells.***

Response: We are grateful for your helpful advice, according to your suggestion, we have drawn a brief diagram for better understanding the hierarchy of the cancer stem cells (Page 33, Figure 2, red ink).

3. ***In the section 3, the knowledge from basic research and clinical research should be divided in order to make them clear.***

Response: Thank you for your constructive comment. According to your suggestion, we have made some relevant modifications in the revised manuscript (Page 14, Line 16 and

Page 15, Lines 15-16, red ink).

4. ***Squares are spreading everywhere. Please use proper font.***

Response: Thank you for your careful checks. We are sorry for this issue and have modified the format and font as you requested.

To Reviewer #2

Reviewer's code: 00013146

Comments to the author:

1. ***As alluded to in the Introduction, the cell of origin of HCC remains uncertain. Although, there is one school of thought, which suggests that all HCCs are derived from progenitor cells, there are also studies suggesting that HCCs may be derived from mature hepatocytes. In the latter scenario, features of "stemness" may represent de-differentiation rather than cell of origin. Although a consideration of HCC histiogenesis is not the main aim of this review article, some consideration of this issue might nevertheless be appropriate.***

Response: We are grateful for your helpful advice. On the basis of your suggestion, we have added some content regarding HCC histogenesis in the revised manuscript (Page 6, Lines 6-9, red ink).

2. ***Some of the markers discussed (e.g. CD44, ICAM-1) appear to have properties primarily associated with more aggressive behaviour rather than specifically being features of "stemness."***

Response: Thank you for your helpful comment; we completely agree with your point.The recently described hepatocellular carcinoma (HCC) with "stemness" is characterized by the expression of markers associated with hepatic stem/progenitor cells in at least 5% of the tumor [1]. Numerous candidate markers have been suggested for this subset of HCCs, including CD44, ICAM-1, and others, and these tumors have been associated with an aggressive behavior and poor prognosis [1, 2]. CD44 is a widely known

cancer stem cell marker in various cancers and has been validated to function in tumor growth and survival and in its aggressive behavior. Cao et al. found that liver cancer stem-like cells (LCSCs) derived from hepatoma cell line MHCC97H acquired stemness traits, such as persistent self-renewal, extensive proliferation, chemoresistance, and overexpression of stem cell markers /cancer stem cell-related proteins (Oct3/4, OV6, EpCAM, CD133 and CD44). In another study, CD44 was found to be preferentially expressed in a CD133+ population in four HCC cell lines, including Huh7, SMMC-7721, MHCC-LM3 and MHCC-97L. $CD133^{+}CD44^{+}$ cells exhibit enhanced abilities to form tumors, are chemoresistant and express a higher level of "stemness"-associated genes compared with their $CD133^{+}CD44^{-}$ counterparts [3]. Intercellular adhesion molecule 1 (ICAM-1) is believed to be responsible for HCC metastasis. Previous studies have shown that ICAM-1 is expressed on hepatocytes in cancerous areas but not on hepatocytes in noncancerous areas [4]. Although it is known that CSCs have crucial roles in cancer relapse and metastasis, whether ICAM-1 is expressed on HCC CSCs remains unclear. Liu et al. indicated that ICAM-1 is a marker of HCC stem cells in humans and mice. ICAM-1 inhibitors slow tumor formation and metastasis in mice. $ICAM\text{-}1^{+}$ cells in blood samples of patients with HCC correlated with aggressive behavior and worse clinical outcomes. ICAM-1 expression is regulated by the stem cell transcription factor Nanog. The concept of "stemness" is still under debate, and one must always cautious about using the term "stemness", which may seem to imply that the tumor cells have stem cell functions. In fact, some research suggests that HCCs that express markers related to "stemness" may represent more advanced HCCs with increased aggressive behavior, persistent self-renewal, extensive proliferation, chemoresistance, overexpression of stem cell/ cancer stem cell markers (such as CD44, ICAM-1 etc.). In conclusion, HCCs with "stemness"-related marker expression were characterized by increased aggressive behavior compared to conventional HCCs.

Reference

1. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 0.1016/S0140-6736(11)61347-0]

2. Zhu PP, Yuan SG, Liao Y, Qin LL, Liao WJ. High level of intercellular adhesion molecule-1 affects prognosis of patients with hepatocellular carcinoma. *World J Gastroenterol.* 2015; **21:7254**-7263 [PMID: 26109813 DOI: 10.3748/wjg.v21.i23.7254]
3. Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, Bussolati B, Camussi G. Isolation and characterization of a stem cell population from adult human liver. *Stem Cells* 2006; **24**: 2840-2850. [PMID: 16945998 DOI:10.1634/stemcells.2006-0114]
4. Liu S, Li N, Yu X, Xiao X, Cheng K, Hu J, Wang J, Zhang D, Cheng S, Liu S. Expression of Intercellular Adhesion Molecule 1 by Hepatocellular Carcinoma Stem Cells and Circulating Tumor Cells. *Gastroenterology* 2013; **144**: 1031-1041 [PMID: 23376424 DOI: 10.1053/j.gastro.2013.01.046]

3. No mention is made of biliary epithelial markers such as CK7 or CK19, which are often used as markers of a progenitor cell phenotype in HCC.

Response: Thank you so much for bringing this to light. We have made some relevant supplemental additions in the revised manuscript (Page 6, Lines 14-16, red ink).

It is a well-established theory that human hepatocellular carcinomas (HCCs) usually emerge during the process of multi-step carcinogenesis. Normal mature hepatocytes transform into pre-malignant lesions and then well-differentiated early stage HCCs (early HCCs) arise from these pre-malignant lesions [1]. Following recent studies of hepatic stem cells or progenitor cells, however, some HCCs are now thought to emerge from hepatic stem or progenitor cells. Carcinomas of combined type (combined hepatocellular and cholangiocarcinoma) and cytokeratin-positive HCCs (such as CK7 and 19) are now recognized as examples of such alternative carcinogenic pathways [2]. Several types of cytokeratins are components of the cellular skeleton and are expressed in liver tissues, in which CK7 and CK19 are referred to as a cytokeratin pattern of bile ducts, as they are expressed only in the bile duct epithelium rather than liver cells. Interestingly, abnormal expression of cytokeratin is found in some parenchymal hepatic cells, whose phenotype is similar to that of bile duct cells in pathological conditions, where both CK7

and CK19 are expressed. Similar to the simultaneous expression pattern of cytokeratins in hepatocellular carcinoma, CK7 and CK19 are expressed in progenitor cells during liver occurrence [3,4]; thus, the conclusion isdrawn that CK7 and CK19 may serve as relatively specific markers of liver cancer stem cells (LCSCs), playing significant roles in hepatocellular carcinoma.

Reference

1. Roskams T. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. Oncogene 2006; 25:3818-3822 [PMID: 16799632]
2. Durnez A, Verslype C, Nevens F, Fevery J, Aerts R, Pirenne J, Lesaffre E, Libbrecht L, Desmet V, Roskams T. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology* 2006; **49:**138-151.
3. Kim H, Choi GH, Na DC, Ahn EY, Kim GI, Lee JE, Cho JY, Yoo JE, Choi JS, Park YN. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. *Hepatology* 2011; **54**:1707-1717 [PMID: 2205674 DOI: 10.1002/hep.24559]
4. Van Eyken P, Sciot R, Desmet VJ.A cytokeratin immunohistochemical study of cholestatic liver disease: evidence that hepatocytes can express 'bile duct-type' cytokeratins. *Histopathology* 1989; **15**: 125-135 [PMID: 2476370]

We have also addressed the steps listed in the scientific editor's email：

Step 1. Please revise your manuscript according to the reviewer's comments.

Response: Manuscript has been revised according to reviewers' comments, and highlighted the corresponding changes.

Step 2. Please update the manuscript according to the Guidelines and Requirements for Manuscript Revision-Minireview.

Response: Manuscript has been update according to guidelines and requrements for minireview.

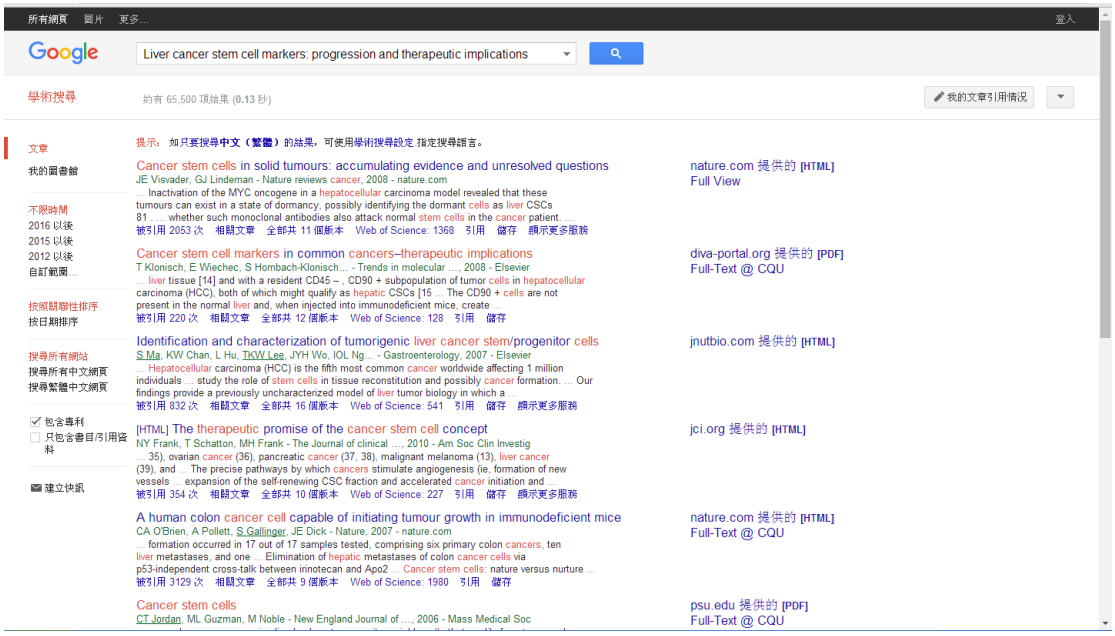
Step 3. Please provide an Audio Core Tip.

Response: An audio core tip has been performed and submitted.

Step 4. Please subject the manuscript to CrossCheck analysis and the final title to Google Scholar search, and store screenshot images of the results.

Response: Our Institution is not subscribed to Crossref, for this reason we are not able to perform a check using this program.

The final title has been checked using Google Scholar (see attached screenshot).



Step 5. Please provide the files related to academic rules and norms. The files related to academic rules and norms include the conflict-of-interest statement.

Response: All the authors declared no conflict of interest. The signed copy of the conflict of interest was provided along with the revised manuscript.

Step 6. Please provide the approved grant application form(s) or funding agency copy of any approval document(s)/letter(s).

Response: This file has been provided.

Step 7. Please revise the language of your manuscript.

Response: The revised manuscript was edited by Elsevier Language Editing Services.

Step 8. Please sign the Copyright Assignment form.

Response: The copyright assignment form signed by all the authors are attached as PDF file along with the revised manuscript

Step 9. Submit the revised manuscript and all related documents.

Response: The revised manuscript and all related documents have been submitted.