October 31, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13633-revised to reviewers' comments.docx).

Title: The chemokines and their receptors play important roles in the development of hepatocellular carcinoma

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Name of Journal: *World Journal of Hepatology*

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We firstly want to give our sincere thanks to the reviewers for their comprehensive analysis of our manuscript. The critical comments are very helpful for improving the quality of our review. We have fully addressed the comments and revised our manuscript according to them. We now provide our point-by-point responses to all the comments as detailed below.

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

Sincerely yours,
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Reviewer 02860712

Comments #A): *I would add several tables (see the cartoon in Balkwill FR J Pathol 2012) aimed at summarizing the chemokine systems which are implicated in HCC oncogenesis , tumor progression, and immune escape. This comment applies in particular to the first section of the review (pages 3 and 4) where the Authors enlist the chemokines of relevance in HCC. All these molecules have been associated with the clinical outcome. However, the role played by each axis in cell survival, angiogenesis and leucocyte recruitment are different . For the sake of clarity, this issue should be summarized in a table and should represent the introduction to the "special" sections of the review.*

Response: We have added a table to summarize the functions of chemokines involved in HCC regarding the categories discussed in this review (Table 2). In addition, we have constructed a concise cartoon to illustrate the core concepts in this review (Figure 1).

Comments #B) *Why the sequence of the section is EFFECT ON IMMUNE FUNCTION, MIGRATION AND INVASION OF HCC, GROWTH AND SURVIVAL OF HCC, ANGIOGENESIS, INFLAMMATION? As the natural history of HCC is characterized by the transition from normal liver to chronic hepatitis, cirrhosis, hepatocellular carcinoma and tumor progression, I would follow this sequence.*

Response: We have revised the sections mentioned in the following sequence: INFLAMMATION, EFFECTS ON IMMUNE CELLS, ANGIOGENESIS, and DIRECT EFFECTS ON BEHAVIORS OF HCC CELLS.

Comments #C)*:-Is there a relationship between the genetic and epigenetic alterations which lead to HCC and the expression of distinct chemokine axes? Is there any relationship between the recently published molecular signatures of HCC outcome and intra-tumoral expression of chemokines? In this regard, it is of interest that Hoshida et al (NEJM 2008) found, among the significant survival genes defined in non-tumoral tissue, CCL21, CXCR4 and CCL19.*

Response: It is quite interesting to explore the relationship between the genetic and epigenetic alterations and the expression of distinct chemokine axes. There have some studies focusing this topic besides the paper by Hoshida et al. (NEJM. 2008). Chang et al. find CXCL12 1–3′A gene polymorphism is a factor related to an increased susceptibility to the risk of HCC (Clin Chem Lab Med. 2009). Yeh et al. also demonstrate that the genetic polymorphism of CCR2-64I increases the susceptibility of HCC (J Surg Oncol. 2010). Interestingly, Tsai et al. discover that CCL5-28, CCL5-403, and CCR5 genetic polymorphisms increase susceptibility to HCC; the polymorphisms of these chemokines also manifest a strong synergic effect with alcohol and tobacco consumptions (Med Oncol. 2012). These data demonstrate the importance of alterations of chemokine genes; however, the detailed mechanisms are still lacking. Furthermore, to our knowledge we don't find the published data unveiling the epigenetic alterations of chemokines in HCC. For this topic, more research are still ongoing and we think the overall evidence at present is not enough to give us a clear landscape, and therefore we don't include this section in our review. Of course we believe this topic is indispensible in future studies of HCC.

Comments #D)*:- The text needs some minor language polishing.*

Response: We have edited the manuscript and corrected the spelling and grammar mistakes.

Reviewer 02860775

Comments- *Chemokine effects on HCC cells, including migration, invasion, growth and survival, should be all incorporated in one, single chapter.*

Response: We have incorporated these parts into the single section DIRECT EFFECTS ON BEHAVIORS OF HCC CELLS.

Comments - *The table proposed by the authors is quite generic in the current shape, and should be more focused on the topic covered by the review. My suggestion is to include a table summarizing for each chemokine of interest, the pro and anti-tumorigenic effects in HCC as they relate to the different functional effects (homing of immune cells, inflammation, angiogenesis, cancer cell activities, …) and then, highlighting those amenable of therapeutic interference.*

Response: We have added a table to summarize the specific functions of the different chemokines involved in HCC according to the topics of the review (Table 2).

Comments - *Iconography is absent at all and at least a figure should be included to facilitate the understanding of the general reader. I suggest a micrograph showing histological chemokine expression in HCC, or a cartoon addressing the most relevant paracrine and autocrine mechanisms regulated by chemokines in HCC.*

Response: We have constructed a concise cartoon to illustrate the core concepts in this review (Figure 1).

Comments - Writing presents some errors (typos, spelling) throughout the text; a fluency improvement by a native English speaker is recommended.

Response: We have edited the manuscript and corrected the spelling and grammar mistakes, and we have also sent our manuscript to a professor from USA for further improvement in language.

Comments *1. The role of macrophages in HCC and their characterization as M1 and M2 phenotypes should be better outlined, for instance in the paragraph dealing with "effects on immune cells", since this issue is then recalled as target of the chemokine-based combination therapy. A recent review on this topic (Sica A, Hepatology 2014) may be of help.*

Response: We have re-analyzed the articles relating to macrophages in HCC and added a brief description of M1, M2 and M2-like phenotypes according to the review recommended.

Comments *2. Reference #87 is unclearly stated. It is also incorrect to consider NAFLD as a model of liver disease progressing to HCC, as instead, it is the case for NASH.*

Response: We have re-analyzed this article and corrected our misunderstanding of NAFLD as the precedent disease to HCC. According to the original article, CXCL1, 2, and 16 directly increase the proliferation of HCC cells at different concentrations in vitro (VanSaun et al. PLOS ONE. 2013. Figure 7); therefore, we retain the citation of this article in the section DIRECT EFFECTS ON BEHAVIORS OF HCC CELLS.

Comments *3. The relevance of CD133+ cells secreting CXCL8 as tumor-initiating cells in HCC should be better addressed (see ref. #96).*

Response: We have given more descriptions on $CD133^+$ tumor-initiating cells (TICs) and added necessary citations. The angiogenic functions of CXCL8 derived from $CD133^+$ TICs have been explained with more details according to the original article.

Comments *4. In page 14, the sentence "the oval cell response is partially ameliorated ..." is quite*

vague and ambiguous: it must be clarified.

Response: We have clarified the ameliorated oval cell response according to the original article and added necessary information about oval cells.