

January 28, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6210-revised.doc).

Title: Roles of Tregs in the development of hepatocellular carcinoma: A meta-analysis

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6210

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer 1 (02445140):

(1) However only manuscripts written in English should be included, being impossible for the reviewer to verify the reliability of papers written in other languages.

We sincerely appreciate the reviewer's suggestions. It is indeed a question that among the 23 studies eventually met the predefined inclusion criteria and entered the meta-analysis, 17 articles were written in the form of Chinese. The exact reasons contributed to such a status in the scientific research area concerning the relationship between Tregs and HCC in human were not entirely

clear to us. In order to ensure the effect of the meta-analysis, we decided to include all the 23 studies. We also expect more and more studies written in English to provide precious data.

Reviewer 2 (02861148)

- (2) The fact that the frequency of circulating Tregs and Tregs in the HCC tumor tissue was increased in HCC patients compared to healthy controls respectively the non-tumoral liver tissue does not necessarily mean that Tregs play a significant role in the pathogenesis of HCC**

We completely agree to the reviewer's comments. We have replaced the sentence "Tregs play a significant role in the pathogenesis of HCC" into "There is a significant association between the high-expression of Tregs and the development of HCC" (first sentence of the last paragraph in the Discussion Section: marked by underline and blue).

- (3) Does an overexpression of Tregs correlate with time to progression, overall survival, or more aggressive tumor characteristics (e.g. metastasis, vascular invasion, higher tumor grading etc.)?**

23 enrolled studies were focused mainly on the frequency of circulating or tumor tissue-special Tregs between healthy and HCC patients. Only a few articles was found to analyze the relationship between Tregs and the other tumor or patients states such as time to progression, overall survival, metastasis, higher tumor grading etc. Based on those limited data, we had performed the subgroup analyses and the results revealed no obvious positive associations.

(4) The manuscript lacks a detailed discussion. The authors only superficially discuss the role of Tregs (in HCC) and their potential role in the development/progression of HCC. I am also missing a critical review of the current status and implications for future studies/research.

We greatly appreciate the consultant's suggestions. In the revised version in discussion section, we have further added some content with regard to the review of current status and implications according to the reviewer's recommendation (The third and fourth paragraph and last four sentences of the fifth paragraph in the discussion section-marked by underline and blue).

Reviewer 3 (02861333)

(5) In the abstract, both the circulating and the tissue populations of Tregs among HCC patients was found to be higher than healthy controls, but there are three OR. Which OR refer to circulating and the tissue populations of Tregs?

Sorry for our negligence. Now, we have changed the sentence as "Frequency of circulating Tregs in HCC patients was 87% higher than the healthy controls (OR=1.87 95%CI: 1.49-2.34); Frequency of Tregs in HCC tumor microenvironment was significantly higher than that in tumor-surrounding tissue and biopsies of healthy livers (OR=4.04 95%CI: 2.10-7.79, P=0.000; OR=2.869 95%CI: 2.16-3.82, P=0.000 respectively), the P-value were all less than 0.01." (the second and third sentence of the RESULT part in the Abstract section-marked by underline and blue).

(6) Recent studies have showed regulatory T cells display heterogeneous functions. Although the population is the same, the functions could be different. So, briefly discuss the related progression in the discussion section.

We greatly appreciate the reviewer's comments. A briefly discussion has been made corresponding to the reviewer's recommendation in the revised version (The third and fourth paragraph and last four sentences of the fifth paragraph in the discussion section-marked by underline and blue).

(7) Explain the reason for the cutoff value of 5cm for tumor size and 20 ng/ml for AFP

This meta-analysis was made based on the data of 23 enrolled studies focused on the frequency of circulating or tumor tissue-special Tregs between healthy and HCC patients. Only limited articles were performed to investigate the relationship between Tregs and the other tumor or patients states such as tumor size and AFP levels. In 23 articles, only 5 studies were found to provide data concerning the association between Tregs and tumor size. In the mentioned 5 articles, 5 cm was chosen as a threshold for small or large liver tumors. As for the AFP levels, data from only 3 studies were available, and 20 ng/ml in the 3 article were chosen.

(8) What about the survival in high Treg patients or tumor tissue compared with low Treg patients or tumor tissue.

We sincerely appreciate the reviewer's suggestions. We also believe the survival comparison between high Treg patients and low ones is more valuable.

However, it's a pity that the current studies did not involve such objective in their initial design, so the association about above information could not be assessed in the present meta-analysis. Nevertheless, we believe that such content would be an important aspect to be complemented in the further study.

(9) Redundant introduction. Please refine.

Reversion of the introduction has been made in the revised manuscript without changing of the general idea.

Furthermore, we have carefully checked our manuscript and revised some awkward sentences.

3 References and typesetting were corrected

We sincerely hope that the revised manuscript meets your approval and the approval of the reviewers, and that it is now acceptable for publication in your esteemed journal.

Thank you again for your consideration of our manuscript in the *World Journal of Gastroenterology*

Sincerely yours,

A handwritten signature in black ink, reading "Hong-qiang Zhao". The signature is written in a cursive, flowing style.

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