June 4, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10806-edited.doc).

Title: High expression of CCL20 is a poor prognosis factor by enhancing proliferation and migration of hepatocellular carcinoma cells through epithelial-mesenchymal transition

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated
All formats on the manuscript have been corrected according to the 'Format for highlighted contents' and 'Format for original articles'.
2 Revision has been made according to the suggestions of the reviewer
(1) Reviewer 1
Question 1: It has been reported previously that high expression of CCL20 is associated with poor prognosis in patients with hepatocellular carcinoma after curative resection (PMID: 22072303).
Response: The paper PMID:22072303 aimed to investigate the prognostic significance of CCL20 in patients with HCC after curative resection. Our study not only detected expression of CCL20 in HCC tissues and analyzed its prognostic significance for HCC patients, but also further analyzed the possible mechanisms underlying CCL20 might promote EMT-like phenotype via PI3K-AKT and Wnt pathway.
Question 2: The experiments conducted to explore the mechanism of CCL20 were using a recombinant protein rather than an expression vector. Without the product information or the catalog number, it's hard to evaluate whether this recombinant CCL20 could represent the actual functions in vivo/vitro. More importantly, since these products usually provided together with a carrier protein or in special solution, acutely this experiment was not designed strictly due to the lack of a proper control experiment. Accordingly, the conclusion would be suspicious.
Response: I apologize for our neglect on writing and provide the lot number at the corresponding position. In this study we focus on CCL20's effect on HCC cells and specimens. So we only design the experiments with CCL20 and without CCL20 treated HCC cells. According to the reviewers advice, we will further investigate the synergies between different chemokines and their receptors which closely associated with tumor progression (eg. CXCL8, CCR6).
Question 3: The authors tried to demonstrate that CCL20 could promote EMT phenotype via PI3K-AKT and Wnt pathway, but this conclusion was only drew from the correlation analysis between the CCL20 and PI3K/Wnt-related protein expression by immunohistochemistry assay. Actually, PI3K and Wnt pathways themselves are

frequently activated in the advanced or metastatic HCC, so the coincidence that the high expression of CCL20 and those pathway proteins can not give confirmative evidence of their regulation relationship.

Response: To our knowledge, even with advance HCC not all HCC cells present abnormal activation of signaling pathway due to the heterogeneity of HCC cells. We first divided HCC specimens into high-CCL20 and low-CCL20 group according to the expression degree,then discuss the possible correlation with abnormally activation of signaling pathway proteins.

Question 4: EMT-like phenotype is a very tricky trait during the cell culture; it could appear transiently or with cell passages in practice. A stable transition could be confirmed by wound healing experiment and the switch between E-cadherin and N-cadherin.

Response: Besides the morphological changes we identified EMT-related protein (E-cadherin and vimentin) by western blotting (Fig 3A) and migration ability by transwell assay (Fig 2B) in order to evaluate the role of CCL20 in promoting invasion and metastasis of HCC cells via EMT-like change.

(2) Reviewer 2

Question 1: In fig 1, they should show the almost same lesion of HCC tissue. Therefore, the reader can easily compare the expression of these proteins in the same tumor.

Response: In fact we use the serial sections to detect different indicators, non-canerous liver tissues in the same slice as control. The typical figures we chose in fig1 only with the purpose of comparing the expressing difference of indicators between HCC tissues and adjacent non-canerous liver tissues.

Question 2: In fig2, they should find the HCC cell lines that highly expressed CCL20. Moreover, the knock down analysis should be carried out since the importance of CCL20 expression for the proliferation and migration activity of HCC were not clear.

Response: According to the reviewers advice, we will further investigate the synergies between different chemokines and their receptors which closely associated with tumor progression (eg. CXCL8, CCR6).

Question 3: The other chemokines might also contribute to the proliferation and migration activity of HCC. They should analyze or discuss the importance of CCL20 in comparison to other chemokines.

Response: Thank you for reviewer's helpful advice, in this manuscript we found the phenomenon that CCL20 induced EMT in HCC cells. In order to elaborate the possible mechanisms we will further investigate by design siRNA of CCL20 and AKT or WNT with the purpose of making the results more convincing.

(3) Reviewer 3

Question 1: The Kaplan–Meier is for estimating the survival function from lifetime data. It is often used to measure the fraction of patients living for a certain amount of time. Cox regression results in estimates of how much the predictor increases or decreases the odds of the event occurring and whether time to event is increased or decreased. Cox regression may be suitable for estimating the relationship of the OS or RSF and the clinicopathologic characteristics.

Response: We used Kaplan-Meier analysis the survival time of patients with HCC, CCL20 expression and the clinicopathological characteristics were evaluated using the chi-square test. Univariate analyses for faotors of OS and RSF were performed by using the chi-square test and the log-rank test, respectively (REFERENCES: PMID: 22072303). The representation is not accurate due to mine lack of statistics knowledge. Deeply apologize again.

Question 2: Describe the information and the titer of antibody product used in every experiment.
Response: We have added the details at the corresponding position.
Question 3: The knock-down analysis and gene transduction should be carried out to clarify the importance of CCL20 expression for the proliferation and migration in HCC.
Response: Thank you for reviewer's helpful advice, in this manuscript we found the phenomenon that CCL20 induced EMT in HCC cells. In order to elaborate the possible mechanisms we will further investigate by design siRNA of CCL20 and AKT or WNT with the purpose of making the results more convincing.

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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