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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5478-review.doc).

Title: Expression of MCP-1/CCL2 in gastric cancer and its relationship with tumor hypoxia

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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 reviewers

   (1) Wu et al. (2013) showed that serum CCL2 was significantly correlated to age and N stage. Whether the author's results have consistency?

   Our research mainly focused on the expression of CCL2 in the postoperative specimens, but Wu *et al* focused on the serum CCL2. In our paper, the expression of CCL2 in the tumor sections was correlated to the AJCC stage which was consistent with the results in Wu's paper. However, the relationship between CCL2 expression level and age showed no statistical significance in our research.

   (2) Have CCL2 lower expression in normal parts of gastric cancer tissue?

   CCL2 protein was mainly expressed in cytoplasm of tumor cells. The expression of CCL2 was lower in normal parts of gastric tissue than its expression in tumor section. It could be visualized in the revised Figure 2. (the legend of Figure 2 in page 6, and the revised Figure 2 in page 16)

   (3) Authors should provide a better explanation of the tumor and sample characteristics shown in figures 1 and 2. According to figure 1, CCL2 and HIF-1a expression increases from section a to section c, where no expression is observed, and this seems to correspond to histological differences in the tissue sections analyzed. Is this related to different hypoxia degree in these sections? The main characteristics of these sections should be described in the text.

   Thank you for your advice. We have amended the figures and provided better

explanation. (Page 6, 7, 16)

In our research, the consistency of spatial distribution of CCL2 and HIF-1a expression indicated that CCL2 expression was relevant with tumor hypoxia. The relationship of different hypoxia degree in these sections with the expression of CCL2 was not quite clearly in the analysis.

(4) A better image of tumor samples could be provided. It is difficult to recognize as tumor cells, the cells observed in section a, figure 1. Also for sections b and c, though these are not so clear to correspond to tumor tissue.

Thank you for your advice. In order to differentiate the tumor tissue and normal parts, the original figures have been replaced. (Figure 1)

(5) A description of tumor characteristics would also be appreciated in figure 2. Are D, E and F images amplified sections of A, B, and C, or do they correspond to other samples?

In the original paper，the D, E and F images are the amplified sections correspond to A, B and C.(Figure 2 in the original paper) However, we replaced the original images. A better image of tumor samples has been provided to illustrate the CCL2 expression level more clearly in tumor tissue.

The revised figure 2 showed expression pattern of CCL2 in human gastric cancer tissue by means of immunohistochemistry. (A): Expression pattern of CCL2 in gastric cancer (100×). (D, E): Expression pattern of CCL2 in gastric cancer (400×). The D and E images are the amplified sections correspond to B and C. (B, D): Negative staining for CCL2; (C, E): Positive staining for CCL2. (Details were showed in the results in page 6, and the revised figure in page 16.)

(6) Authors state that both tumor cells and stromal cells were observed to express CCL2, but there is no clear image showing this.

Thanks very much for the suggestions. The expression of CCL2 in stromal cells was much weaker than that in cancer cells. I'd like to provide a better image to state CCL2 expression in tumor cells and stromal cells. (Figure 2, page 16)

(7) English writing needs review, particularly the results and discussion sections. The last sentence of the 'survival analysis' paragraph reporting table 2 results, is not clear. ,

The last sentence of the 'survival analysis' paragraph is a bit confusing. We have amended the description in the paragraph. In a multivariate Cox regression model, the

results showed the CCL2 expression was an important prognostic indicator in gastric cancer. And the result was consistency with the AJCC stage, which was a prognostic factor in cancer. The p values of the two factors in the table showed statistically significance. ("Survival analysis" paragraph in Page 7)

(8) Bibliography should be updated, since both CCL2 and HIF-1a have been reported to be expressed in gastric tumors.

The references have been updated.

(9) In Results, the authors described "Follow-up time was censored if the patient was died or lost to follow-up." Dead patients should be treated as failure case, not as censored cases. If dead patients were treated as censored case in the statistical analysis, the Kaplan-Meier survival curve should be redrawn, and the log-rank test and Cox proportional hazard model should be corrected. What is first, HIF-1alpha or CCL2? Why? Is CCL2 expressed in some selected gastric cancers or in every gastric cancer tissue with hypoxia?

Thank you for your advice. The description of "Follow-up time was censored if the patient was died or lost to follow-up." did not clearly define in the paragraph. The patient who was died of other cause rather than gastric cancer should be censored. The description has been corrected. ("Survival analysis" paragraph in Page 7)

Because of the heterogeneity in individual gastric cancer patient, CCL2 expressed in selected gastric cancer with hypoxia. We hypothesize that hypoxia might be a driving factor of CCL2 expression. However, the more detail relationship between CCL expression and hypoxia would be studied in our group.

(10)It should be discussed why CCL2 expression did not show significant association with well-known prognostic marker, such as AJCC stage and tumor size. Please check if the HR value for AJCC stage Ⅰ / Ⅱ vs. Ⅲ/Ⅳ(3.242) is correct.

We have discussed CCL2 might be a valuable predictive marker of gastric carcinoma, as it was correlated with the AJCC stage of tumor. We have checked the HR value for AJCC stage Ⅰ / Ⅱ vs. Ⅲ/Ⅳ.There is no mistake. So I keep the data of the paper as it is original.

3 .References and typesetting have been corrected.

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

Sincerely yours,

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