February 10, 2013

Dear Editor:

We would like to thank you and the reviewers for your careful consideration of our manuscript entitled '**Disruption of interstitial cells of Cajal networks after massive small bowel resection**' (ESPC Manuscript NO: 1494). We have carefully addressed reviewers' concerns and believe that the revised manuscript has been significantly enhanced. Our point-by-point comments follow.

We appreciate your time and consideration of our manuscript for publication in *World Journal of Gastroenterology*. If you have additional concerns, please feel free to contract me.

Sincerely,

Jie CHEN and Wei CAI

Response to Reviewers

Major revisions

1. I cannot find in the Materials and Methods or the Results at which post-operative day tissues were collected from control sham-operated animals. It is absolutely crucial in order to draw valid results that all following comparisons are done between SBS and control animals of the same post-operation day. Please provide clearly this information. –

In our study, bowel tissues of Group A (sham) and Group B were harvested at day 7 post-surgery to avoid the potential influence of surgical procedures on the experimental findings. We have included that information in the revised manuscript.

2. Was there a negative control in the immunohistochemistry staining? How was the quantification performed? Please explain.

In the study, negative controls for immunohistochemical staining were performed. We have only provided a representative figure for the Reviewers. The quantification was performed by the terms of strong, medium, and weak immunopositivity.

3. A better explanation is required for the statistical analysis about the number of observations for all experiments. In most cases it is not mentioned. When it is mentioned, does n refers to number of animals, or number of tissue samples from the same animal, or number of observation from the same tissue sample? Please provide details.

In the beginning, 30 rats were divided randomly into three experimental groups (n=10, each). Each rat underwent bowel transactions and reanastomosis. After surgery, 9 rats in Group A, 8 rats in Group B and 7 rats in Group C survived. At day 7 post-surgery, tissue samples were collected from the 9 rats in Group A and 8 rats in Group B rats. At day 14 post-surgery, tissue samples were collected from the 7 rats in Group C. Afterwards 7 tissue samples of each group were chosen at random for electrophysiological studies, and 4 tissue samples of each group were chosen at random for immunohistochemistry staining and Western blot analysis. We have provided detail in the manuscript where necessary.

4. In the immunohistochemistry results, images are not at all clear. Are there no positive cells at all in the SBS1W photograph? Please explain in text the type of staining you observe, cytoplasmic or membrane?

In the immunohistochemistry staining, a small number of positive cells were present in the SBS1W group. Both the cytoplasm and membrane were stained, which is consistent with the information sheet provided with the antibody against c-Kit protein.

5. About the Western blotting experiments. It seems to me that total smooth muscle tissue was homogenized and total protein was used for the Western experiments. This tissue however, we already know from the immunohistochemistry results, that contains less c-kit positive Cajal cells in the SBS1W group, so it is expected that c-kit and mSCF will be lower,

as it is corrected for total GAPDH. I therefore believe that we cannot conclude alterations in the mSCF/c-kit signaling pathway, unless changes are shown per ICC cells, and/or functionally.

We agree with the concerns of the reviewer. In our study, the ultrastructure morphology of ICC, phenotypic changes in ICC and subsequent altered electrophysiological functional activity in the ileum after mSBR were observed. These findings indicated the possible involvement of mSCF/c-kit signaling. However, our findings do not conclusively demonstrate the association between mSCF/c-kit signaling pathway and the changes observed in ICC cells. Future studies are required to clearly elucidate the signaling mechanisms.

Minor revisions

1. Please make sure you present the full explanation of acronyms the first time they appear in the manuscript. For example ICC on the 'aim' section of the Abstract.

Correction has been made in the revised version

2. Abstract line 4: instead of mSCF/c-kit that imply the estimation of a ratio, use c-kit and mSCF protein content as it was actually measured.

Correction has been made in the revised version

3. Abstract line 5: Delete the phrase : 'The ICC and associate….' as it is not fully justified.

We have delete this sentences in the revised version.

4. Introduction: Is it 'adaptation' or 'adaption'? Please be constant.

Correction has been made in the revised version

5. Page 7, line 19, delete 'with'

We have delete the word ‘with’ in the revised version.

6. Page10, line 10. Please explain how did you separate smooth muscle tissue.

The bowel was opened along the mesenteric border and the lumenal contents were washed away with KRB. Segments of the bowel were pinned to the base of a Sylgard silicone elastomer dish and the mucosa was removed by sharp dissection. The remaining tunica muscularis was used for electrophysiological recordings and Western blotting.

7. In figure 2, put lettering in every subcellular structure you describe, to make it easier for a non-specialist reader to understand.

Correction has been made in the revised version

8. Page 13, line 21: ‘… had changed slightly’. Please be specific. What type of changes, increase or decrease. If no statistical significance can be shown, there were no changes…

Correction has been made in the revised version

9. Figure 3. Slow waves lacked a secondary component in SBS1W rats. Please correct.

Correction has been made in the revised version

10. Page 14, line 5. Please delete ‘ICC are … tunica muscularis’ as it repeats somehow the Introduction.

We have delete this sentences in the revised version.

11. Figure 4. What does ** mean? The Figure shows statistical significance in both SBS1W and SBS2W groups, which however conflicts what is described in the text.

We appreciate the comment and have corrected Figure 4 in the revised manuscript. The figure shows that the protein expression levels of mSCF

and c-kit were significantly decreased with mSBR in the SBS1W group and the protein expression levels returned to baseline compared to sham-operated rats by day 14 after mSBR.