ANSWERING REVIEWERS

April 2, 2013

Dear Editor,

Please find enclosed the edited manuscript and my response to the reviewer's comments.

Title: Neurotensin Receptor 1 Overexpression in Inflammatory Bowel Diseases and Colitis-Associated Neoplasia

Author: Gui X , et al.

Manuscript No: Ms.wjg/2013/1326

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:
   1) *"Abbreviations are not always followed by their full explanation as currently requested"* – Revised accordingly in the attached updated version.
   2) "*Statistical analysis was performed by Fisher's test. Since the compared groups were more than two (see table 2: negative, +1, +2 and +3) an analysis of variance could have been more appropriate*" – Agreed. The statistics was done again by ANOVA test, and the same statistic significance was found.
   3) *"It is unclear the site of NTSR1 expression: epithelium. stromal cells, lamina propria or all these locations?"* – We have made it clear in the Material & Methods and results sections (pages 8 and 9) that our analysis focused on the colonic mucosal epithelial cells, but did not include the lamina propria or stromal cells.
   4) *"The system of expression assessment which was used by the authors is almost unusual. A more accurate semiquantitative marker of a molecule in immunohistochemistry is the labeling index, i. e. the percentage of positive cells. The count of positive cells in intestinal specimens is performed on 10 well-oriented crypts for the epithelium and on randomized fields containing at least 1000 cells in the stroma. Is there a specific reason which induced the authors to avoid the use of conventional count methods?"* – Instead of counting thousands of cells to get the positivity rate (index), which is a very tedious and inefficient way, we used the way that every practicing pathologist uses in our daily work, i.e., to semiquantitate the immunostain results into negative, 1+, 2+, and 3+, based on the combination of both intensity of the stain and the estimation of positive cells. This method is more popular in clinical practice as well as in pathology research literature. Please see our description in the Material and Methods section (page 9).
   5) *"Figure 1 A does not seem to be a haematoxylin-eosin staining"* – The reviewer is correct. Fig 1A is not H&E staining but also an immunostaining for NTSR1. We hope to include both low and high magnifications of the immunostaining in normal mucosa. Due to the limitation of the space, we do not

put a H&E micrograph. Besides, the readers can easily tell the normal mucosa from the immunostain slides.

6) *"NTSR1 was not expressed in all cases of colitis, dysplasia and cancer. Have the authors a hypothesis to explain the lack of expression in some inflammatory, pre-cencerous and cancerous lesions? This aspect could be a good topic for the Discussion".* – Good point. Thanks to the reviewer's advice. We do not have the explanation, but we tried to add a little discussion there. Please see Discussion section (page 12).

3. References and typesetting were corrected.

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

Sincerely yours,

Xianyong Gui, MD, PhD