

Ya-Juan Ma
Science Editor
Editorial Office
World Journal of Gastroenterology

April 2, 2015

Dear Editor Ma,

I enclosed the revised manuscript entitled "**Toll like receptor-4 activation upregulates cystathionine β synthetase expression through an NF- κ B-dependent mechanism in a rat model of chronic visceral pain**" by Yuan et al. for consideration for publication in World Journal of Gastroenterology.

First of all, we thank you and the reviewers for the valuable comments and suggestions. We have gone through the manuscript carefully and made corrections, as suggested. In addition, we have performed 2 additional experiments. The results have been added into the manuscript as supplementary information (Supplementary Figure 1 and Figure 2).

The detailed responses to the reviewers' comments are included in this letter on the separated page. Changes in text are highlighted in RED.

Thank you for your time and consideration. We look forward to your responses.

Sincerely yours,

Guang-Yin

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Reviewer 1-Comments

This manuscript tried to explain the development of CBS in IBS from the aspect of TLR4/NF- κ B pathway, which is quite a novel angle to explore IBS. However, I'm very sorry to say it's not a well-written article and the authors failed to make their point very clear. The work is valuable, but I would like to suggest the authors to improve the article from the following aspects:

>> Thank you, the reviewer, for the comments.

1. There are lots of grammar errors to correct;

>> We have corrected the grammar errors. In addition, the manuscript has been edited by the professional editing company (AJE). The certificate has been uploaded.

2. The current introduction part is a little bit too long and not focused enough;

>> Thank you. The introduction part has been shortened.

3. The authors missed some points when presenting the experiment design, such as the number of animal and basic information of the chemicals and instruments used in the study;

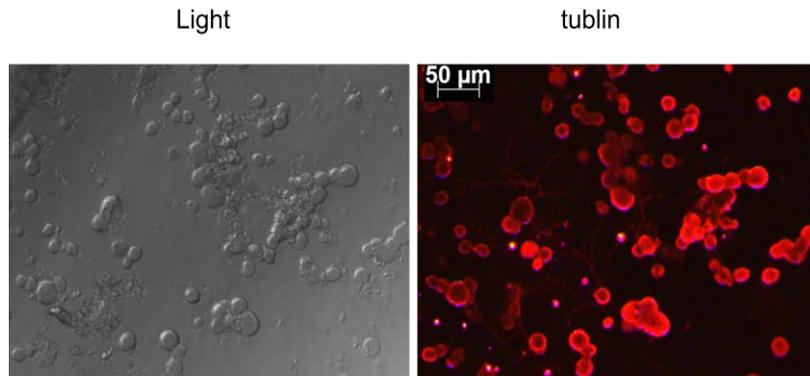
>> Thank you for the comments. The number of animals and basic information of the chemicals and instruments used in the study have been added into the manuscript.

4. The figures are clear enough to tell the results if only the authors could explain the comparison relationships beneath the figures, instead of telling all of the results again in the context.

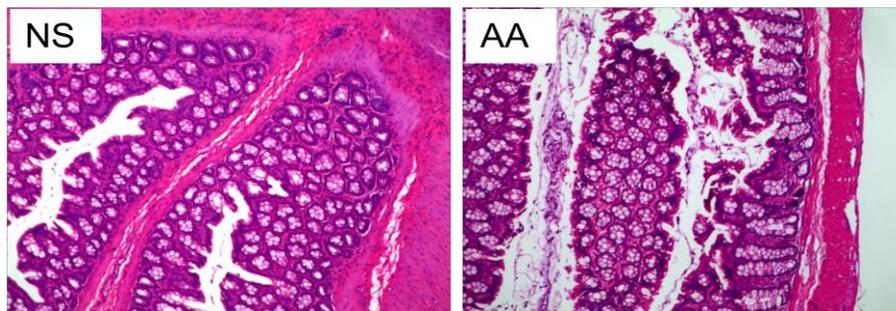
>> We agree with the reviewer. Some results have been deleted in figure legends to avoid repeat.

5. Cells used in PCT and cell culture were not confirmed by any markers, which would influence the accuracy of further measure as there are some other cells in the suspension of DRGs after digestion. Therefore, more information about cell marker should be present;

>> Thank you. We have performed additional experiments to demonstrate the cells in cell culture were labeled by tubulin, which is a mark for neural staining (Supplementary figure 2).



6. Pathological pictures should be provided to exclude inflammatory alterations.
 >> We have performed additional experiment to show that the inflammatory alterations were not observed in the colon of rats at the age of 7 week (Supplementary figure 1). This is consistent with previous studies (Winston et al, 2007)



Reviewer 2-Comments

The manuscript by Yuan and colleagues entitled ‘Toll like receptor-4 activation upregulates cystathionine β synthetase expression through an NF-kB-dependent mechanism in a rat model of chronic visceral pain’ is well written and presents an array of interesting data on the role of neuronal TLR4 in the development of acetic acid-induced visceral pain in an NFkB dependent manner. The English is of a good standard but would benefit from some polishing. The paper is technically sound and the findings are interesting. A number of issues should be addressed as indicated below:

>> Thank you, the reviewer, for the comments.

Check for a number of typos through out the manuscript - would benefit from review by a native English speaker.

>> We have corrected typos through out the manuscript. In addition, the manuscript has been edited by the professional editing company. The certificate has been uploaded.

Restrict description of methods to the methods section (remove from results).

>> The description of methods has been removed from the result section

The results should be described more concisely. Similarly, the figure legends could be more concise – no need to repeat information in the figures again in the text.

>> We agree with the reviewer. The repeated information in the figures has been removed from the text.

The findings on hydrogen sulfide (H₂S) signaling and its endogenous synthesizing enzyme cystathionine- β -synthetase (CBS) in an animal model of chronic visceral hypersensitivity may not translate to the clinical scenario of IBS and shouldn't be overemphasied.

>> Thank you. We have modified the manuscript to avoid overemphasizing the statement.

The authors should speculate as to the mechanisms by which acetic neonatal acid treatment to increase TLR4 expression in DRGs. What is stimulating this PAMP in these neurons?

>> In this study, we focused on the mechanisms by which CBS expression was upregulated. We agree with the reviewer that we should speculate as to the mechanisms by which neonatal acetic acid treatment increased TLR4 expression in DRGs. Although the stimulating mechanisms in these neurons are not clear, we speculate that cell damage/injury and oxidative stress might be the causes to increase TLR4 expression in DRGs. This has been added in the discussion section.