



February 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6916-review.doc).

Title: "Epidermal growth factor up-regulates serotonin transporter and its' association with formation of visceral hypersensitivity".

Author: Xiu-fang Cui, Wei-mei Zhou, Yan Yang, Jun Zhou, Xue-liang Li, Lin Lin, Hong-jie Zhang*

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6916

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

Reviewer1:

(1) Please do not have abbreviations in the title.

Thank you for the nice suggestion. We have modified the title.

(2) Please add the study rationale to the introduction; how do you put the low levels of EGF and visceral hypersensitivity together and design this project.

Thank you for the valuable suggestion. We added some information to the introduction to provide evidences as connecting low levels of EGF and visceral hypersensitivity.

(3)The paper lacks a study hypothesis; you need to add it to the introduction section.

We have added the study hypothesis to the introduction section.

(4) In the introduction, you need to briefly describe the findings of the study.

Thank you for the nice suggestion. We added the findings of our study in the introduction section.

(5) Current study models of visceral hypersensitivity are required to be described in the introduction.

We have added some contents about visceral hypersensitivity model in the introduction section.

(6)Some spots of English errors need to be edited; such as “a blinded pathologist” ; n=10 each.

We have corrected the errors in the revised paper and the manuscriptr been edited and proofread by BIOMEDITOR.

(7) The cell culture condition is not correct.

Thank you for the nice comment. We have corrected.

(8) The Western blotting procedures can be simplified.

Thank you for the nice suggestion. We have simplified the procedures in the revised paper.

(9) How did you calculate the qRT-PCR results?

We have added some information about calculating the qRT-PCR results in method section.

(10) the “T test” should be “t test”

Thank you for the nice suggestion. We have corrected.

(11) The findings need to be verified by human data; this reviewer suggests collect blood samples from IBS patients to see if the levels of EGF are lower than healthy subjects.

Thank you for the valuable suggestion. We detected the plasma level of EGF in IBS patients and added the result in the revised paper.

(12) what does the sentence mean, “Each point represents the mean of 10 rats from 3 independent experiments” in figure 1 legend.

We have modified the figure1 legend.

(13)Figure 1-3 legends need to be re-organized.

Thank you for the nice suggestion. We have re-organized the figure 1-3 legends.

(14) The description of the statistical results of figure 4 is quite confused; please re-word the whole legend.

Thank you for the nice comment. We re-organized the figure4 legend.

(15)As if you used vehicles in control group in figure 4 and figure 5; it is not a proper control agent. Please carry out the experiments again using an irrelevant protein as controls.

Thank you for the nice comment. In the figure4 and 5, the vehicles=control, as treatment with EGF group.

Reviewer2:

In this manuscript, Cui et al aimed to investigate the changes in epidermal growth

factor (EGF) levels in a visceral hypersensitivity rat model, and if EGF regulates serotonin transporter (SERT) expression and function. The authors show that EGF levels (determined by ELISA) are decreased in colon tissues and plasma of rats with visceral hypersensitivity. Also, serotonin transporter (SERT) expression is decreased both at protein and mRNA levels (Western blot and real time PCR, respectively) in these animals with respect to controls. They also show that EGF-treatment induces SERT expression through EGF receptor (EGFR) in rat intestinal crypt cells. It is already known that EGF up-regulates SERT gene expression in intestinal cells and that SERT expression and function are down-regulated in colon and rectal tissues of patients with irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). However, this research could be important because it is still uncertain whether the decreased levels of EGF can lead to SERT down-regulation and ultimately to visceral hypersensitivity, which is a characteristic of IBS.

Major comment:

1. Authors entitled their manuscript as follows: “Transcriptional regulation of SERT by EGF affects development of visceral hypersensitivity” . However, their results don’t demonstrate that EGF (through EGFR) levels modulate visceral sensitivity regulating SERT expression and function. They only used the Pearson's correlation analysis and say that there is a correlation between EGF and SERT levels in colon tissues. Thus, authors should better confirm their hypothesis. If they inhibit EGFR function (with a blocking antibody or a pharmacologic inhibitor) in vivo, do SERT levels in colon decrease? Do these treated-rats develop visceral hypersensitivity? Also, authors could use dominant-negative EGFR point mutation mice (used in reference No. 20) to test the hypothesis that the EGFR signaling pathway is involved in SERT expression and visceral hypersensitivity.

Thank for the reviewer’s valuable comments. We agreed reviewer’s comments. Our study has some limitations. In our study, we demonstrated that EGF down-regulated SERT-mediated 5-HT uptake into enterocytes by the in vitro experiment, and there was the correlation between EGF and SERT levels in colon tissues from rats with visceral hypersensitivity using the Pearson's correlation analysis, and deduced that EGF down-regulated SERT-mediated 5-HT uptake maybe involved in the development of visceral hypersensitivity. We didn’t confirmed the findings of EGFR signaling pathway on SERT expression and change of visceral hypersensitivity in vivo. Future studies are needed to use dominant-negative EGFR point mutation mice to test that the EGFR signaling pathway is involved in SERT expression and visceral

hypersensitivity.

Minor comments:

2. References No. 24 and 26 are the same.
3. Almost half of the references were published 15 or more years ago. Authors should update the references list.

Thank for the reviewer's nice suggestions. We re-organized the references and updated the references.

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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