

Format for ANSWERING REVIEWERS



Jan 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7721-revised.doc).

Title: Cx32 and Cx43 promoter methylation in *Helicobacter pylori*-associated gastric tumorigenesis

Author: Yu Wang, Li-Hua Huang, Can-Xia Xu, Jing Xiao, Li Zhou, Dan Cao, Xiao-Min Liu, Yong Qi

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7721

First, one contributing author (Xiao-Min Liu) is added at author line, address, contribution paragraph, attached BPG Copyright Assignment. In the acknowledgements, another support "Changsha Municipal Science & Technology Project (No. K1106036-31)" is added. **These along with other revisions by ourselves and English professional are marked blue.**

The AIM in the Abstract is reduced, but the whole abstract has 280 words (**in the "Format for original articles": no less than 336 words**).

The manuscript has been improved according to the suggestions of reviewers with answers as follows (**with the revision words marked red in the manuscript**):

Reviewed by 02570299

Abstract: should be changed to connexin 32 and 43. H.pylori is also Helicobacter pylori.

Answer: "Cx32 and Cx43" in the title and author contribution paragraph has changed to "Connexin 32 and 43". We use "Connexin 32 and 43 (Cx32, Cx43)" for its first appearance in the Abstract, Core tip and Introduction, and use "Cx32, Cx43" thereafter for simple and separate expression of Connexin 32 and 43. We use "Helicobacter pylori (*H. pylori*)" for its first appearance in the Abstract, Core tip and Introduction and "H. pylori" later.

Discussion: The authors conclude that ~~~provides a new target for the demethylation treatment of gastric cancer. What kinds of treatment of gastric cancer will appear in the future? More detailed information is needed.

Answer: We add this in the Discussion: "From the perspective of our research, the treatment of gastric cancer may include the eradication of *H. pylori*, adding DNA-demethylation agent (for example, 5-azacytidine), and overexpression of Connexin 32 and 43".

References: The references are comprehensive and well placed. Tables: Ok.

Reviewed by 02546581

This is a nicely written manuscript about new therapeutic targets in treatment of gastric cancer and its different carcinogenesis stages with H.pylori infection. The analyses seemed to be performed well and are clearly described in the manuscript.

I have some issues to mention:

- The authors should give the exact p-values for each analysis (even in the abstract).

Answer: Yes, now the exact P -values (or $P < 0.001$) for each analysis are given in the text. For describing a group of comparisons, we use " $P < (\text{the largest } P \text{ value in these comparisons})$ ". In Figs. 2 and 3, Tables 4 and 5, $P < 0.05$ (generally-recognized significant difference) for a, b, c, d, or e denotes the P range of several comparisons, so it's not changed.

- Please delete " $P < 0.05$ for most comparisons"; line 5, results part in the abstract. That's not very scientific.

Answer: It has been replaced by "(GC vs NGM: $P < 0.001$ for Cx32, $P < 0.001$ Cx43)", representative of other comparisons between different gastric carcinogenesis stages.

- The authors should give more details about the tumor stages of patients with gastric cancer.

Answer: Yes, we have added the following content inside quotation marks, and the two reference papers are added (and later numbering is changed in references and in the text).

Diagnoses of endoscopy and pathology were made according to the 8th edition of the *Cecil Essentials of Medicine* [11], "Chinese consensus on chronic gastritis [12] and Chinese guidelines for diagnosis and treatment of gastric cancer (2011 Edition) [13]. Generally, the endoscopic findings of NAG included mucosal congestion, edema, or accompanying with little hemorrhage and erosion, and those of CAG included thinning mucous layer, shallowing or disappearing folds, visibility of the submucosal vascularity, and fine granules on the surface. The pathological findings of NAG included necrosis of the superficial mucosal epithelium and infiltration of lymphocytes and plasma cells in the lamina propria, and those of CAG included shrinking gastric glands with the number reducing and shallowing gastric pits. IM was identified that gastric epithelium is replaced by intestinal epithelium, accompanying with the goblet cells secreting acidic mucus, absorptive epithelial cells with striated edge, and Paneth's cells. DYS was identified that atypical cells proliferated but it is not enough to be diagnosed as cancer. GC was identified that the cancerous tissues infiltrate the mucosal, submucosal or entire layers, taking on polypoid, ulcerous, and diffuse infiltrative types".

Reviewed by 02493110

Following previous work of the decrease of Cx32 and Cx43 expression in precancerous lesions and gastric cancer is associated with H.pylori infection, co-culture of gastric epithelial cells with H.pylori reduces the expression of Cx43, and eradication of H.pylori can up-regulate the Cx32 and Cx43 expression in precancerous lesions, Wang et al present their new findings of the hypermethylation of these genes' promoter may be the important mechanism for the decrease of Cx32, Cx43 mRNA expression at gastric carcinogenesis stages with H.pylori infection. Overall the hypothesis is sound and experiments are well-designed and the results are supporting the conclusions; however some statements should be clarified. The specific comments are listed below.

1. The manuscript can be understood but would still profit from proofreading by a native speaker. There are many mistakes in the typewriting, grammar and form of manuscript.

Answer: We've asked an English proficient person to read the manuscript and carefully examined the typewriting, grammar and form. It should be greatly improved.

2. In the whole manuscript, the presentation of abbreviation should be standard.

Answer: "Cx32 and Cx43" in the title has changed to "*Connexin 32 and 43*". We use "*Connexin 32 and 43 (Cx32, Cx43)*" for the first appearance in the Abstract, Core tip and Introduction, and use "Cx32, Cx43" thereafter for simple and separate expression of *Connexin 32* and *43*. Please suggest a better formula, so we can change again. Gene names "CDX2, RASSF1A and P16" are from the citation paper with long original name and now italicized (also in question 4). Another abbreviation "LATS1" is now changed to "large tumor suppressor 1 (*LATS1*)". Also you can see "proton-pump inhibitors

(PPI), or histamine receptor (H₂)”.

3. The resolution and clarity of Figures should be modified and improved. Red wavy lines under letters need to be removed in Figure 2 and 3.

Answer: We checked all the figures. Figure 2A is a screenshot from the Q-PCR software and hard to be very clear. We think other figures are fundamentally OK. Red wavy lines (marking the word spelling error) under letters in Figure 2 and 3 have been removed, and if they appear again in different Word version, we ask the editor to remove them (select, right key, neglect).

4. Page 4, line 10: Gene name “CDX2, RASSF1A and P16” should be standardized and consistent as “Cx32 and Cx43”.

Answer: Gene names “CDX2, RASSF1A, P16” are from the citation paper and now italicized as “*CDX2, RASSF1A, P16(INK4A)*”. Their original names are Caudal Type Homeobox 2, Ras association domain family 1 isoform A, and p16 (inhibitor of cyclin-dependent kinase 4), respectively. If needed, we can add them.

5. Some sentences about results description in Abstract and Introduction repeated in later section of Results and Discussion. Please modify it.

Answer: The results description in Abstract is necessary according to the requirements of the Journal, and in the Results, it is detailed separately and corresponding to the Figures and Tables. The description of our previous clinical studies^[5-7] in the 2nd paragraph in the Discussion part was deleted since it's already stated in the Introduction. The AIM in the Abstract is reduced.

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

Sincerely yours,

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MD

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