ANSWERING REVIEWERS

March 12, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7639-review.doc).

Title: *Helicobacter pylori* infection, gastrin and cyclooxygenase-2 in gastric carcinogenesis

Author: Yun Shao, Kun Sun, Wei Xu, Xiao-Lin Li, Hong Shen, Wei-Hao Sun

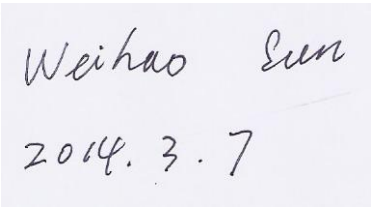
Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7639

Please find attached a revised version our manuscript to be considered for publication in the *World Journal of Gastroenterology* as a review article. We carefully examined the reviewers' comments and revised the manuscript. The manuscript has been improved according to the suggestions of reviewers and editors. We are re-submitting the manuscript here together with an accompanying letter point-to point response to the reviewers' comments. We hope that the revised manuscript would be found acceptable for publication in the *World Journal of Gastroenterology*.

This manuscript has been seen and approved by all authors, who have taken due care to ensure the integrity of the work. The authors warrant full disclosure of any financial and personal relationships with other people or organizations that could inappropriately influence their work. We have also highlighting all changes made in the manuscript to make it easier to follow them.

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



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Response to editor

1. We have checked the grammar and spelling and invited a native English speaker to help edit the manuscript. With all corrections made, we are confident that we can now achieve an A grade of manuscript language quality. As corresponding author and final responsible of this review, I would like to point out that I have authorship in 15 Pub-Med indexed articles written in English and I have never needed or been required by the publishers to make use of manuscript language service by professional English language editing companies.

2. Author contributions and Core tip have been added.

3. References and typesetting have been corrected.

4. The coordinate graphs have been changed to be decomposable (as powerpoint format).

Response to reviewer 1 (2536994)

Comment 1. References should be refreshed to show the recent results of related studies.

Response: Some older references have been replaced by newer editions to show the recent results of related studies. Six references of the original manuscript have been excluded from the revised manuscript as below:

1 Wang RT, Wang T, Chen K, Wang JY, Zhang JP, Lin SR, Zhu YM, Zhang WM, Cao YX, Zhu CW, Yu H, Cong YJ, Zheng S, Wu BQ. Helicobacter pylori infection and gastric cancer: evidence from a retrospective cohort study and nested case-control study in China. *World J Gastroenterol* 2002; **8**(6): 1103-1107 [PMID: 12439934]

2 Welin M, Holmgren NM, Nilsson P, Enroth H. Statistical model of the interactions between Helicobacter pylori infection and gastric cancer development. *Helicobacter* 2003; **8**(1): 72-78 [PMID: 12603619 DOI: 110 [pii]]

6 Fox JG, Rogers AB, Ihrig M, Taylor NS, Whary MT, Dockray G, Varro A, Wang TC. Helicobacter pylori-associated gastric cancer in INS-GAS mice is gender specific. *Cancer Res* 2003; **63**(5): 942-950 [PMID: 12615707]

8 Mulholland G, Ardill JE, Fillmore D, Chittajallu RS, Fullarton GM, McColl KE. Helicobacter pylori related hypergastrinaemia is the result of a selective increase in gastrin 17. *Gut* 1993; **34**(6): 757-761 [PMID: 8314507 PMCID: 1374257]

10 Kargman S, Charleson S, Cartwright M, Frank J, Riendeau D, Mancini J, Evans J, O'Neill G. Characterization of Prostaglandin G/H Synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. *Gastroenterology* 1996; **111**(2): 445-454 [PMID: 8690211 DOI: S0016508596003654

[pii]]

15 Li HX, Chang XM, Song ZJ, He SX. Correlation between expression of cyclooxygenase-2 and angiogenesis in human gastric adenocarcinoma. *World J Gastroenterol* 2003; **9**(4): 674-677 [PMID: 12679908]

We now have added four newest references to the revised manuscript as below:

2 **Greenfield LK**, Jones NL. Modulation of autophagy by Helicobacter pylori and its role in gastric carcinogenesis. *Trends Microbiol* 2013; **21**: 602-612 [PMID: 24156875 DOI: 10.1016/j.tim.2013.09.004]

3 **Long M**, Luo J, Li Y, Zeng FY, Li M. Detection and evaluation of antibodies against neutrophil-activating protein of Helicobacter pylori in patients with gastric cancer. *World J Gastroenterol* 2009; **15**: 2381-2388 [PMID: 19452583 DOI: 10.3748/wjg.15.2381]

13 **Rodrigues NL**, Dore M, Doucet MY. Expression of cyclooxygenase isoforms in ulcerated tissues of the nonglandular portion of the stomach in horses. *Am J Vet Res* 2010; **71**: 592-596 [PMID: 20433387 DOI: 10.2460/ajvr.71.5.592]

17 **Yamac D**, Ayyildiz T, Coskun U, Akyurek N, Dursun A, Seckin S, Koybasioglu F. Cyclooxygenase-2 expression and its association with angiogenesis, Helicobacter pylori, and clinicopathologic characteristics of gastric carcinoma. *Pathol Res Pract* 2008; **204**: 527-536 [PMID: 18462890 DOI: 10.1016/j.prp.2008.01.002]

Comment 2. More evidence should be added to demonstrate the mechanisms of HP-associated gastric cancer, for example the functions of virulent factors of HP.

Response: The main carcinogenic effect of *H. pylori* is in the presence of the cytotoxic associated gene A (cagA) and vacuolating cytotoxin A (vacA)[35,36]. A meta-analysis conducted by Huang *et al*[33] showed that the risk of gastric cancer was twice as high in people who were positive for antibodies against CagA in sera.

To clarify whether *H. pylori* CagA can induce gastrin expression, Zhou *et al*[52] constructed a eukaryotic expression vector pcDNA3.1/*cagA* and a luciferase reporter vector pGL/gastrin promoter, and then co-transfected them into gastric cancer cells, and suggested that CagA could activate the gastrin promoter and up-regulate gastrin mRNA expression in AGS and SGC-7901 cells.

Romano and coworkers reported that *H. pylori* up-regulates COX-2 mRNA expression and stimulates the release of PGE_2 in MKN 28 gastric mucosal cells *in vitro*; and this effect was independent of VacA, CagA, or urease-generated ammonia[87]. However, other *in vitro* studies have demonstrated that *H. pylori* up-regulates COX-2 expression in human gastric cancer cells; and this effect is specifically related

to VacA toxin[88,89].

These contents and related references have been added into the revised manuscript to demonstrate the mechanisms of HP-associated gastric cancer.

Comment 3. The three sections of COX-2 should be concise and clear.

Response: According to the reviewer's comment, we have adjusted the related sections of COX-2 to be more concise and clearer. "The *in vitro* studies have also demonstrated that *H. pylori* up-regulates COX-2 expression and increases PGE_2 synthesis in human gastric cancer cells [68]." "Thus, COX-2 expression induced by *H. pylori* infection is a relatively early event during carcinogenesis in the stomach. Overexpression of COX-2 is related to tumor invasion and lymph node metastasis in the gastric carcinoma." "recently showed that gastrin stimulated expression of COX-2 and IL-8 in the human gastric epithelial cell line AGS, transfected with the CCK-2R." "stability of both COX-2 and IL-8 in a p38-dependent manner but also enhanced COX-2 and IL-8 gene transcription through the transcription factors activator protein-1 (AP-1) and NF-κB, respectively." "In AGS gastric cancer cells, NF-κB was found to regulate COX-2 expression [95]. Inhibition of COX-2, either mediated by NF-κB inhibition or by treatment with non-specific (indomethacin) or specific COX-2 (NS-398) inhibitors, resulted in suppression of cell proliferation [95]. Another transcription factor, AP-1, was found to be" In the revised manuscript, we have excluded these contents.

Response to reviewer 1 (2536589)

Comment 1. New references should be added to introduce the recent development of research. The case in point is a meta-analysis which suggests a protective role for H. pylori infection in the prognosis of GC (PLoS One. 2013 May 7; 8(5):e62440.)

Response: As suggested, this reference has been added to the revised manuscript and the list of References:

37 **Wang F**, Sun G, Zou Y, Zhong F, Ma T, Li X. Protective role of Helicobacter pylori infection in prognosis of gastric cancer: evidence from 2,454 patients with gastric cancer. *PLoS One* 2013; **8**: e62440 [PMID: 23667477 DOI: 10.1371/journal.pone.0062440]

Nevertheless, a later meta-analysis conducted by Wang *et al*[37] showed a protective role for *H. pylori* infection in the prognosis of gastric cancer. Several studies have also examined the relationship between *H. pylori* infection and prognosis of patients with gastric cancer, providing evidence of a better prognosis in patients with *H. pylori* infection compared with patients without *H. pylori* infection[38-41]. The underlying mechanisms need to be further elucidated, which could provide new therapeutic

approaches for gastric cancer. We have added this content in the "**EVIDENCE FOR THE CARCINOGENICITY OF *H. PYLORI* FROM EPIDEMIOLOGICAL STUDIES** " of the revised manuscript.

Comment 2. Add more evidence to clarify the relationship between HP and gastric cancer， DNA demethylation, for example.（Cancer Prev Res (Phila). 2013 Apr;6(4):263-70.）

Response: As suggested, this reference has been added to the revised manuscript and the list of References:

82 **Niwa T**, Toyoda T, Tsukamoto T, Mori A, Tatematsu M, Ushijima T. Prevention of Helicobacter pylori-induced gastric cancers in gerbils by a DNA demethylating agent. *Cancer Prev Res (Phila)* 2013; **6**: 263-270 [PMID: 23559452 DOI: 10.1158/1940-6207.CAPR-12-0369]

On the other hand, aberrant DNA methylation in gastric biopsies from *H. pylori*-infected patients was found to be correlated with a greater gastric cancer risk[76,77]. Previous studies have reported that infection with *H. pylori* is associated with promoter methylation of various gastric cancer-associated genes[78,79] and eradication of the bacteria was able to reverse the process in patients with gastritis, but not in patients with intestinal metaplasia[80,81]. Recently, Niwa and colleagues[82] demonstrated that treatment with the DNA demethylation agent 5-aza-2'-deoxycytidine decreases the incidence of gastric cancers in an animal model of *H. pylori*-promoted gastric cancer. This study also showed that induction of aberrant methylation is an important mechanism for gastric carcinogenesis by *H. pylori* infection. We have added this content in the "**GASTRIN AND DNA METHYLATION POTENTIATES THE CARCINOGENIC EFFECTS OF *H. PYLORI* INFECTION** " of the revised manuscript.