Dear Editor and Reviewers:

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript entitled " *Helicobacter pylori* inhibits the cleavage of TRAF1 via a CagA-dependent manner" (ESPS Manuscript NO: 28286). We appreciate you and the reviewers very much for the positive and constructive comments and suggestions on our manuscript.

Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to us. We have studied your and reviewer's comments carefully and tried our best to revise our manuscript according to the comments. All of the revisions was highlighted yellow in the updated version of the manuscript. Attached please find the revised version, which we would like to submit for your kind consideration.

The main corrections in the paper and the responses to the reviewer's comments are as flowing:

Responds to the reviewers' comments:

Reviewer 1: 00227403

Comment 1: In the abstract, section methods, please specify the acronym "AGS" or clarify "human gastric cancer cell line AGS"

Response: We have put the sentence "human gastric cancer cell line AGS" instead of only "AGS".

Comment 2: In the introduction, sentence "Our previous study showed that TRAF1 is upregulated by H. pylori infection in both gastric epithelial cells and mice." What do you mean? In both cellular and animal model?

Response: In the previous study, we showed that TRAF1 is upregulated in three gastric epithelial cell lines (AGS, GES-1, and SGC7901) as well as in C57BL/6 mice infected with H. pylori.

Comment 3: At the end of the introduction, the authors should delete the sentence "In the present study, H. pylori infection was found to inhibit the cleavage of TRAF1 and inhibit the activation of caspase-8 in the presence of the apoptosis inducer. We also found that H. pylori virulence factor CagA is mainly involved in the H. pylori-mediated inhibition of TRAF1 cleavage. This would increase the relative amount of full-length TRAF1, which would present the antiapoptotic role rather than the proapoptotic role in the H. pylori-infected cells." And replace it with "aim of our work has been…."

Response: Considering the reviewer's suggestion, we replaced this section with "The aim of our work is to elucidate the effect on TRAF1 cleavage regulated by H. pylori and the roles of H. pylori virulence factors regulating TRAF1 cleavage. For better understanding the role of H. pylori infection on TRAF1 cleavage, in the present study we detected the cleavage of TRAF1 in AGS cells co-cultured with H. pylori or sterile saline alone in the presence of apoptosis inducer. We also analyzed the roles of H. pylori virulence factors that may regulate TRAF1 cleavage using isogenic cagA-, vacA-, and cagE-null mutants." We hope this new part would meet with your requirement.

Comment 4: In section RESULTS, TRAF1 is cleaved via caspase-8 in AGS cells infected with H. pylori, the sentence "To confirm the cleavage site of TRAF1 is located at aspartic acid 163 in the 160LVED163 motif, AGS cells were…." should be "To confirm that the cleavage site of…"?

Response: We are very sorry for our inappropriate writing and we have rewritten the sentence "To confirm that the cleavage site of…".

Special thanks to you for your good comments.

Reviewer 2: 03551098

The comment: I recommend this manuscript as a brief article.

Special thanks to you for your good comments.

Reviewer 3: 02941672

Comment 1: The detailed characteristics of AGS cell and the reason why this cell line was selected needs to be explained.

Response: AGS cell, a well-differentiated human gastric cancer cell line, was established from the human gastric adenocarcinoma tissue of one Caucasian women aged 54 years. In our previous study, we showed that the upregulation of TRAF1 in AGS cell was most significant following co-culture with *H. pylori* among three cell lines (AGS , SGC 7901 and GES-1). We think it could be easier to detect and illustrate the role of *H. pylori* infection on TRAF1 cleavage, so we selected AGS cell for our experiments.

Comment 2: The author revealed TRAF1 was cleaved by apoptosis inducer and it was attenuated by caspase-8 inhibitor. Do you have data that caspase-8 itself cleaved TRAF1?

Response: Actually, the previous study has showed that caspase-8 itself could cleave TRAF1 [14], so we further confirmed that using caspase-8 inhibitor.

Comment 3: In Figure 1D, the H.pylori seems to act like apoptosis inducer (TNF-a+CHX). If H.pylori inhibits TRAF1 cleavage, TRAF1-C ought not to appear in 2nd lane from the left.

Response: In our experiment, we actually found that TRAF1 could be slightly cleaved by only *H.pylori* infection but not with apoptosis inducer (TNF-a+CHX). However, under the condition of apoptosis inducer, *H. pylori* infection significantly inhibited the cleavage of TRAF1 compared with the uninfected group. That means *H.pylori* seems to be apoptotic role as well as play an antiapoptotic effect under specific conditions. The confused apoptotic or antiapoptotic functions played by *H.pylori* is also in line with the current studies and need further research.

Comment 4: In Figure 2B, TRAF1-C with *H.pylori* increased cell apoptosis. This is in contradiction with the hypothesis that H.pylori inhibits TRAF1 cleavage, because TRAF1-C production should decrease if H.pylori inhibits TRAF1 cleavage.

Response: like Comment 3, TRAF1-C is not completely inhibited and we actually could detect a few of TRAF1-C production under our experimental condition, especially co-cultured with *H.pylori* for 24 hours.

Comment 5: For easy understanding, correlation diagram in H.pylori and TRAF1 cleavage had better be presented.

Response: Considering the reviewer's suggestion, we made graphs to better present the correlation in *H.pylori* and TRAF1 cleavage in Figure 3, which we hope would be more clear and easy to understand.

Special thanks to you for your good comments.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions. Looking forward to hearing from you.

Yours sincerely,

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