Author's response to reviews

Title: Gene expression analysis of a Helicobacter pylori-infected and high-salt diet-treated mouse gastric tumor model: Identification of CD177 as a novel prognostic factor in patients with gastric cancer

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Author's response to reviews: see over
Reply to the Referee 1:

1. Reference: We additionally cited two papers (Gonda et al., 2012 and Suh et al., 2012) that reported expression of CD177 or MUC13 in the gastric mucosa, and described brief comments in the conclusion of Abstract and Discussion sections. Gonda et al. have performed a microarray analysis suggesting that \( Cd177 \) expression was reduced in the gastric mucosa of \( H. \ felis \)-infected mice with dysplastic lesions by oral supplementation of folic acid. Because they compared stage-matched groups to detect regulated genes only by the treatment of folic acid, it is unclear whether \( Cd177 \) expression is associated with degrees of gastritis and dysplasia. We also replaced the citation for "Global cancer statistics" by Parkin et al. (2005) to the updated version by Jemal et al. (2011).

2. Adenoma: Adenomatous lesions would be helpful for investigation of early gastric carcinogenesis as the reviewer indicated. However, human gastric adenoma specimens were not included in the present study. In addition, the anti-CD177 antibody used in this experiment did not cross-react with rodent tissues. Therefore, we would rather limit the focus of the present study on the prognostic role of CD177 expression in human advanced gastric cancers.

3. Array data: Because the area used for extraction of RNA could not be histologically confirmed for the presence of any lesions, we chose to pool several samples to avoid individual difference. We added a brief explanation about this in Methods section. On the other hand, we added the Venn's diagram for presentation of total results of microarray analysis as Figure 2A. We also corrected the numbers of up- and down-regulated genes, because original version represented the number of probes, not "genes". So every number slightly reduced as a result of the removal of probes overlapping at same genes.

4. Median value: We added the median follow-up period (83 weeks) in the Methods section, as indicated.

5. English: As indicated, the revised manuscript was corrected by a proofreading expert for biomedical article.
Reply to the Referee 2:

1. Animal model: It seems to be still controversial whether *H. pylori* infection is a single cause for gastric carcinogenesis (Fock et al., *J Gastroenterol Hepatol*, 23:351-65, 2008). Previous epidemiological studies and animal experiments have demonstrated that development of stomach cancer is associated with many other factors including salt intake, alcohol drinking and cigarette, containing a wide variety of chemical carcinogen, and that *H. pylori*-induced chronic gastritis also acts as a potent promoter of gastric carcinogenesis. Since not all *H. pylori*-infected people are affected with stomach cancer, these factors may have important roles in determinant of pathogenesis. In the present study, we attempted to mimic the gastric environment of human high-risk group exposed by multiple factors. We added the description about this in Discussion section.

2. Disadvantages: As indicated, the mouse model has not only advantages but also disadvantages such as the instability of cagPAI. We added the brief discussion about the issue. For the number of animals, 21, 5, 15, and 15 mice were assigned to A, B, C, and D groups, respectively, at the commencement of the experiment. However, mice in the group D died or became moribund more than expected. We explained it in the Methods and Results section.

3. Statistics: The statistical procedure used in the original manuscript was inappropriate to evaluate multiple groups, as indicated. We revaluated our data by ANOVA with the Tukey test as post hoc multiple comparison for multiplicity of tumor (Table 2), the Kruskal-Wallis with Tukey test for relative mRNA expression (Fig. 2B), the Chi-square test with Bonferroni correction for tumor incidence (Table 2), and ANOVA or Chi-square test for correlation of CD177 expression and clinicopathological factors (Table 4). The results for mRNA expression demonstrated that expression level of *Muc13* showed a tendency for increase with combination of *H. pylori* and salt, although this was not statistically significant. We totally rewrote the Abstract, Results and Discussion sections based on the revaluation.

4. Scoring: We examined the immunohistochemistry for CD177 as four-grading score (none, weak, moderate and strong) based on the percentage of positive-staining cells.
Because there were no statistical correlation between CD177 expression and any clinicopathological factors including age, histological classification, depth of invasion and lymph node metastasis by the four grade scoring, we limited the description at two categories ("positive" and "negative") in our original manuscript. We added the detail results of the 4-point grading in the Table 4.

5. Scheme: We added the Venn's diagram as Figure 2A to represent the total results of microarray analysis, as indicated. In addition, we corrected the numbers of up- and down-regulated genes in Results section, because original version represented the number of probes, not "genes". So every number was slightly reduced as a result of the removal of probes overlapping at same genes.

6. Legend: As indicated, the explanation for data of real-time RT-PCR was insufficient. We modified the description in the Figure Legend appropriately.

7. Lauren classification and serum: For the classification of the gastric cancer, Lauren’s intestinal and diffuse types correspond to well/moderately differentiated and poorly differentiated types, respectively. We added this explanation in the Table 4. For the serological $H. pylori$ status, we do not have any available serological information of human samples for the present study.

8. Abstract: We rewrote the Abstract to describe the two types of experiment more clearly, as indicated.

9. Ethical vote: We added a description about the ethical approval for investigation using human samples, as indicated.

10. Table and figure: We included all tables in the revised manuscript.