Date: November 15, 2021

To: Dr. Lian-Sheng Ma, Editorial Office Director, Company Editor-in-Chief, Editorial Office

From: Li Dong Wang, MD, PhD, Professor, State Key Laboratory of Esophageal Cancer Prevention & Treatment and Henan Key Laboratory for Esophageal Cancer Research, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China.

Re: Point-by-point response to the comments of the manuscript (ID: 71395), entitled as "Characterization of E-cadherin expression in normal mucosa, dysplasia and adenocarcinoma of gastric cardia and its influence on prognosis".

Dear Dr. Ma,

Many thanks for your kind email of Nov. 8, 2021 for the decision and comments to our submitted manuscript (ID: 71395), entitled as "Characterization of E-cadherin expression in normal mucosa, dysplasia and adenocarcinoma of gastric cardia and its influence on prognosis". Based on the comments and suggestions from the reviewers and editor for the manuscript, all the authors have read and revised the manuscript carefully. All the revisions have been marked in the revised manuscript. The point by point responses are as follow.

Reviewer #1:

1. Material section needs some clarification, with the detailed description of IHC-process, with all materials' cat..no.

Reply: Many thanks for your comments. We have added on paragraph to describe the IHC-process in detailed and the Cat. No. has been added in the section of Materials

and Methods in our revised manuscript on page 7.

2. Were there any paires samples involved into the study: I mean normal, dysplastic and cancer tissues from the same patient? Or if you had bigger slides where dysplastic and/or normal mucosa was present together with cancerous tissue? Did you see decrease in E-cadherin staining even in this contex? Or you did not have samples like this?

Reply: Yes, we do have the matched tissues from the same patient with normal, dysplasia and cancer lesions (Fig1, 2). But, in our study, our experiment was performed with the tissue microarray and these different lesions were from the cancer and adjacent tissues far from the tumor. The tissue microarray we used with a diameter of 1.5mm could not contain the normal and dysplasia and cancer tissue of the same patient.

Fig.1 Fig.2

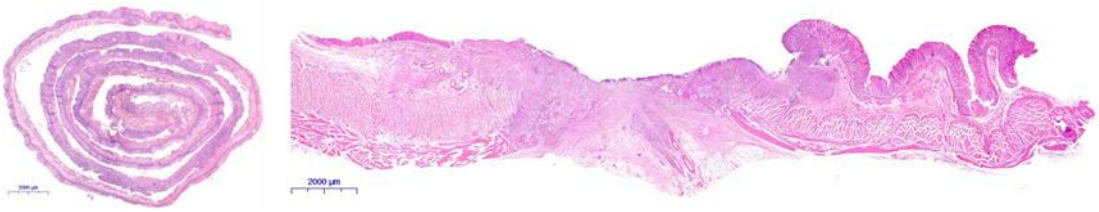

Fig.1 Swiss roll embedded slide contained normal, dysplasia and cancer tissue from the same patient

Fig.2 Big slide contained normal, dysplasia and cancer tissue from the same patient

3. For me the results describe E-cadherin as a differentiation related marker. Was there significant difference in E-cadherin expression when different grades of tumors were compared? Any graph on that? Well, I saw graphs on this, but this was less mentioned in manuscript. Could you please mention and comment it in your masuscript? I think, we could handle E-cadherin as a kind of differentiation marker.

Reply: We agree with you that E-cadherin was as a molecular biomarker of differentiation, as the declined tendency from well differentiation to moderate differentiation to poor differentiation (92.5 vs. 85.4 vs. 82.5%, $\chi 2 = 14.259$, $P =$

0.001). We have added the discussion about the result in the discussion section, on page 11.

4. You mentioned that male patients showed weaker expression of E-Cadherin. Was it because males have higher grades and stages of tumor?

Reply: We have analyzed the correlation between gender and differentiation and stage of GCA, and have found no significant difference in differentiation ($\chi 2 = 1.308$, $P = 0.520$) and stage ($\chi 2 = 0.036$, $P = 0.849$), respectively.

5. Some further minor points: "The staged of patients with GCA were based on the 8th edition" rather staging. "group with negative lymph node metastasis" rather patients without LN metastasis.This sentence appear on multiple sentences. "As we know, the present study is the first report about the E-cadherin protein expression in the lesions progressed from normal gastric cardia mucosa to dysplasia and GCA, and the largest sample study of the expression of E-cadherin protein and its influence on survival with GCA." I would put references here. "there was few reports" plural " It is showed that, in our study, " rather our study showed that…. "negative expression" rather decreased or lacking expression. Again on multiple sentences.

Reply: Thanks so much for your kind comment. We have added related references and modified based on your suggestion correspondingly in our revised manuscript in red paint.

6. "Another interesting finding in the present study was that positive expression of E-cadherin protein in GCA patients at an early stage was higher than in those at an advanced stage (92.3% vs. 83.6%, P = 0.001), which indicates that E-cadherin protein may be a potential biomarker for early warning for GCA." how? E-cadherin expression should be preserved in normal mucosa…I do not get the point here.

Reply: Many thanks for your kind comments. Yes, dysplasia should be considered as early lesion for gastric cardia carcinogenesis, but not early stage of GCA. We have modified this point as follows: Another interesting finding in the present study was

that positive expression of E-cadherin protein in GCA patients at dysplasia lesion was higher than in GCA stage (93% vs. 84.1%, $P = 0.003$), which indicates that E-cadherin protein may be an early potential biomarker for gastric cardia carcinogenesis.

7. "Lastly, we found that in the GCA patients without lymph node metastasis, positive expression of E-cadherin protein indicated better survival than negative expression." I think it might be again the question of grade! Any data, analysis on this? "

Reply: Yes, it was also correlated with degree of differention. Our data showed that, of the patients with negative lymph node metastasis, the patients with E-cadherin protein positive expression had a higher rate ($\chi 2 = 13.060$, $P = 0.001$) of well differentiation than those with E-cadherin protein negative expression. The multivariate analysis of survival showed that both E-cadherin protein expression and differentiation were the independent risk factors for the prognosis of GCA ($P = 0.026$, $P < 0.001$, respectively).

8. "The difference in E-cadherin expression can further stratify the prognosis of patients with negative lymph node metastasis, indicating that E-cadherin protein expression may be a promising prognostic biomarker for non-surgical GCA patients." How do you imagine this.

Reply: In clinical practice, although the overall survival of GCA patients with negative LN metastasis is longer than that of patients with positive LN metastasis, some of the patients still have poor survival, even worse than that of patients with positive LN metastasis. Until now, we still can't predict who has worse survival for the patients with negative LN metastasis. The result of our study will provide us a potential biomarker that can predict accurately the prognosis of these patients with negative LN metastasis, and make it possible for us to optimize the treatment strategy and to improve the survival rate of patients with GCA.

9. I have a concern about the decrease in E-cadherin expression. The decrease is quite

small: G1 92% vs, G3 84%. Was it decrease in focal expression, or was it expression or lack of expression, so something which could be dichotomized?

Reply: As you are concerned, the staining intensity and staining area were under consideration to evaluate the protein expression rate of E-cadherin comprehensively in the materials and methods section of our manuscript on the second paragraph of page 7.

Reviewer #2: Only remark is to this section: reference 12 is published in 1994. I believe that a more up to date literature should be used. "Histopathological diagnosis Histopathological diagnoses for normal mucosa, dysplasia and adenocarcinoma of the gastric cardia were made according to established criteria [12]. Methods used are standard for clinical practice, and for scientific paper it might be a little more complex.

Reply: Thanks so much for your kind concern. We have updated the reference in our revised manuscript, the reference 12 on page 16.

Science editor:

The study analyzed the expression of E-cadherin in GCA and its correlation with patients' outcomes. E-cadherin had been reported to be associated with numerous malignant entities. This study showed similar findings but confined to GCA. Number of comments raised by reviewers' should be answered. The intensity of IHC in figure 1 seems to be no much difference. Besides, it is better to show similar direction of section for Figure 1E, 1F with other IHC figures.

Reply: Many thanks for the concerns. We have replaced the figures the intensity can be distinguished (figure 1). Figure 1A, B and 1C, D are HE and IHC of normal and dysplasia tissue, which show the normal epithelial structure and its polarity. Figure 1E and 1F are HE and IHC of cancerous tissue, which lost the normal epithelial structure and its polarity and replaced by disarranged cancerous tissue.

Best wishes,

Yours sincerely,

Li-Dong