Author's response to reviews

Title: Primary small cell carcinoma of the esophagus: clinicopathological study of 44 cases

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Author’s response to reviews:

Dear Ms. Cherry Battad,

Thank you for your recent letter regarding our manuscript. We have carefully studied the comments and suggestions from the reviewers, and have revised our manuscript accordingly. The following paragraphs summarize our revisions in response to the reviewers’ comments.

Responses to Reviewer ’s (Jian Ming Xu) comments,
1. Issue regarding the follow-up time and the median survival time of studied patients: The median follow-up time for all patients was 11.1 months (3-84.9 months). We have added this information in the Results on paragraph 1 page 6.

2. Issue regarding the figure captions: We have change the accompanying verb "were" to "was" in figure captions of Fig 3.

3. Issue regarding the consistency of abbreviations: We have ensure the abbreviation of Orphan G-protein coupled receptor (Lgr5) is consistent now.

4. Issue regarding the Figure number: We have added the figure number to all figures.

Responses to Reviewer ’s (Rupert Langer) comments,
1. Issue regarding the definition of SCCE: In our study, the diagnosis of SCCE was confirmed by the pathologists of our cancer center combining the morphological features of small cell carcinoma and the expression of neuroendocrine markers. We check the the patient pathology reports again and
found the reports showed all patients have positivity for CgA or/and Syn expression. In addition, expression of other neuroendocrine markers (NSE, CD56) was reported. We provide more clear information by adding some texts on paragraph 1 page 4.

2. Issue regarding the English language corrections: We are very sorry for any inconvenience caused when you were reading our manuscript. Now we have used a professional language editing service to help us to edit the manuscript.

3. Issue regarding the link between SCCE and Lgr5: Lgr5 expression had been found in neuroendocrine tumors and esophageal cancer. A C Iuga reported that LGR5 is a novel immunohistochemical marker for gastrointestinal neuroendocrine tumors. 88% of the primary (3/4 gastric, 23/24 intestinal, 13/14 pancreatic, 0/2 appendiceal) and 87% of the metastases stain positive for cytoplasmic LGR5. LGR5 is positive in the majority of the cases expressing chromogranin and synaptophysin (34/38). On the other hand, Burkhard HA von Rahden had reported that LgR5 was found expressed in 35 of 41 (85%) esophageal adenocarcinomas with Barrett's Esophagus and in 16 of 19 (81%) without Barrett's Esophagus. Laren Becker also found Lgr5 was found to expressed in early esophageal squamous cell. At the basis of the above findings, our study examineed Lgr5 expression in small cell carcinoma of esophagus. We have addressed this issue in discussion part (paragraph 2 page 7).

4. Issue regarding the gene expression of lgr5 with additional RT-PCR: We only achieved paraffin embedding tissues and we could not successfully extracted RNA from these tissues since the RNA was degradation from a long time storage. So we could not conduct additional RT-PCR. In addition to Lgr5, We have examined other stem cell markers, such as CD44. However, the expression is rare and had no correlation with any clinicopathological features of patients.

5. Issue regarding percentage of SCCE: According to the clinical database of our hospital, from 1994 to 2012 at Sun Yat-sen University Cancer Center, there were 5379 cases of esophageal cancer and among them there were 93 (1.73%) cases of SCCE and 44 cases had paraffin embedding tissues. From 1994 to 2012, our hospital had 2061 cases small cell carcinoma and 4.5% were SCCE. We have added some texts in the introduction part(paragraph 1 page 3).

6. Issue regarding the conclusion: we have modified the conclusion part.

7. Issue regarding the cut off for IHC set: The median of the final scores was 4, 47.7% cases had the scores above 4 and 52.3% cases had scores less than or equal to 4. So we consider high expression was defined as the final score above 4 and low expression was the score equal to or less than 4, relatively.

8. Issue regarding the results: we had added the result that high Lrg5 levels are associated with inferior chemotherapy response in result part.

9. Issue regarding the Fig2: we added some texts in figure captions to illustrate the figures were from the representative slides.

We thank the constructive comments and critiques of the reviewers, whose suggestions have helped us to strengthen this work. I hope we have satisfactorily addressed all the issues, and thank you for considering our revised manuscript.
for publication.

Yours sincerely,

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