Author’s response to reviews

Title: Analysis of EGFR, HER2, and TOP2A gene status and chromosomal polysomy in gastric adenocarcinoma from Chinese patients

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Author’s response to reviews: see over
Dear editors,

Thank you very much for your email about our manuscript (MS: 4555040261914632), we have carefully read the suggestions from you and reviewers, and try to answer the questions point-by-point.

- Major Compulsory Revisions

**Question 1.** It is still not entirely clear about the study samples.

1.1 If the 100 samples were just what the authors could get that fit the selection criteria (e.g., intact clinical data, tissues available for 20 slides), during that period of 2000-2005 (see the response letter), then it is really not a “randomly” selected sample as the author noted on page 6, 2nd paragraph.

1.2 Overall, the inclusion and/or exclusion criteria were not clearly described in the texts and would require improvement. In addition, (if there were really some kind of selection process) it should include the information on the TOTAL number of patients that underwent surgery for gastric adenocarcinoma during the study period (and/or the proportion of patients that eventually to be included in the study out of the total of number of patients underwent surgery) should be included.

1.3 And the concern about whether there are potential biases associated with the fact that selected samples were patients with larger tumor size that large enough to make 20 slides (therefore patients with smaller tumor size were not selected for study) is not yet addressed. For example, one can evaluate the biases by comparing patient demo and tumor characteristics between selected and unselected samples. If this can not be done (e.g., data were not available), then the report should at least acknowledge the potential selection biases as a limitation of the study (if it is biologically plausible).

All these should be better explained and described in the texts.
Answer: We agree with the reviewer’s opinion. “randomly” was deleted from our manuscript in “Material and Methods” section. We made corrections about sample selection in “2.1 patients” section, and all corrections have been incorporated in the texts.

“More than half of the patients seen at the Department of Surgery in the Peking Union Medical College Hospital were not Beijing residents, they came from different regions of China. We selected the specimens from our archive paraffin embedded blocks. All the specimens were selected by two pathologists and only those patients whose clinical data intact and the blocks were enough to be cut into 20 slides were selected. One hundred Chinese patients with gastric adenocarcinoma who underwent surgery at the Department of Surgery, Peking Union Medical College Hospital were randomly selected from 2000-2005” were replaced as “The specimens were selected from archive paraffin embedded blocks in Peking Union Medical College Hospital by two pathologists, 793 Chinese patients with gastric adenocarcinoma who underwent surgery at the Department of Surgery, Peking Union Medical College Hospital were eligible to be selected for the period of 2000-2005. Only those patients whose clinical data (include diagnosis, age, sex, address, disease history, etc) were intact and the blocks were enough to be cut into 20 slides were selected, patients from different areas of China were included. One hundred Chinese patients with gastric adenocarcinoma were finally selected in this study. For the small number of patients and specimens with larger tumor size were selected in this study, the significance of this study maybe limited. Because no previous data about Chinese patients, we tried to present our data for reference. If sample size increased, the results would be more representative.”

Question 2. The issue of multiple comparisons is not yet addressed and incorporated in the actual texts. If the Bonferroni adjustment was made to adjust for multiple of 10 tests (should it be 13 tests?), then the p-value that to be considered statistically significant will be 0.005, rather than the stated 0.05 on page 10, 2nd paragraph. Currently it shows “Differences at P < 0.05 were considered significant”. In all the following texts in the Results section wrt p-value and significance should also be
checked for consistency and accuracy. That is, you will only claim a significant association when p<0.005. The authors should provide a sufficient explanation if they do not wish to adjust for multiple comparisons. Other alternative adjustment method can be considered if the Bonferroni correction is thought to be too conservative for the purpose of the study.

Again, all changes/revision should be incorporated in the texts.

**Answer:**

a. In order to avoid concept misunderstanding, we deleted the comparison between EGFR protein expression and EGFR disomy and non-disomy (low trisomy, high trisomy, low polysomy, high polysomy and amplification), so 19 comparisons were performed in our report, and we made some corrections as following. We corrected the descriptions in “2.4 Statistical analysis” section according to reviewer’s suggestion, Bonferroni adjustment was made to adjust for 19 tests of comparisons, so the differences at P < 0.0026 (0.05/19) were considered significant. All the p-values and significance were also checked for consistency and accuracy, and have been corrected in the text, tables and note for tables.

b. We deleted all the descriptions about the comparison between EGFR protein expression and EGFR disomy and non-disomy. In “Abstract”, “EGFR overexpression correlated significantly with EGFR aneusomy.” was deleted.

In the “Results”, “3.2 EGFR status in gastric carcinoma” section, “Sixty-eight percent of the cases showed EGFR aneusomy (non-disomy), and” and “, but was significant associated with EGFR aneusomy (p < 0.05)” were deleted.

In the “Discussion” section, 2nd paragraph, “but EGFR aneusomy showed a significant association with EGFR overexpression. Taken together, these results suggest that EGFR aneusomy may be responsible for EGFR overexpression, although further studies will be required to confirm this hypothesis. Our results showed that EGFR FISH-positive status did not correlate with EGFR overexpression, further studies will be required to confirm their relationship.” were replaced as “Our results
showed that EGFR FISH-positive status did not correlate with EGFR overexpression, further studies will be required to confirm their relationship.”

Table 1 were modified, and the note “#: the association between EGFR protein expression and EGFR disomy and non-disomy (low trisomy, high trisomy, low polysomy, high polysomy and amplification) (P<0.01)” was deleted.