Author's response to reviews

Title: Irradiation-induced telomerase activity and gastric cancer risk: a case-control analysis in a Chinese Han population

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Version: 3 Date: 10 May 2010

Author's response to reviews: see over
May 10, 2010

Dr. Diana Marshall
Scientific Editor
BioMed Central

Dear Dr. Diana Marshall

Thank you for your letter regarding our manuscript (MS. 2329246262903538). I would also like to thank the reviewers for their time and the suggestive comments. We have revised our manuscript and addressed all the questions raised by the reviewers. The point-by-point discussion of each issue is provided below. The detailed changes are underlined in the text of the revised manuscript.

I hope that our revised manuscript is now acceptable. If there are any questions, please feel free to contact me: phone, 86-29-8477731, and email, Guoqiang@fmmu.edu.cn. Thank you very much.

Sincerely,

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Reviewer comments:
The authors have made a considerable effort to address the comments of the reviewers. Many issues have been dealt with satisfactorily but a few outstanding items remain.

Major Compulsory Revisions:
Question 1. Page 2. Conclusions
I feel that the conclusions stated in the abstract are too strong and not supported by the work described in the manuscript. The results of these experiments are hypothesis-generating rather than conclusive. The authors demonstrated an increased rate of radiation induced telomerase activity in Gastric cancer cases and this “may be” associated with gastric cancer risk or development.

Response: We agreed the reviewer’s comments and made related revision on Page 2 as follows.
“Overall, our findings for the first time suggest that the increased $\gamma$-radiation-induced telomerase activity in PBLs might be associated with elevated GC risk. Further confirmation of this association using a prospective study design is warranted.”

**Question 2. Page 4. Paragraph 1.**

“Acturally (sp), the level of $\gamma$-radiation induced telomerase activity is only a biomarker for the evaluation of the inherited inducibility of telomerase activity, but not truly clinically relevant disease”. In this single sentence the authors are responding to several comments from the 2 reviewers….First: because the blood samples were drawn after diagnosis the authors cannot state whether the observed increased telomerase activity was present prior to cancer development and therefore involved in cancer risk or whether it is a systemic biomarker of gastric cancer. Second: is the observed increase in telomerase activity clinically significant/relevant These are two important weaknesses in this study and its design and directly affect how the results should be interpreted. I do not think that combining these two issues into the above statement accurately addresses the problem. For starters…increased telomerase activity “may be” a biomarker….I am not sure that the authors have adequately responded to these issues. I would suggest a more comprehensive series of statements on these issues in the discussion section of the manuscript would be appropriate (possibly on page14 paragraph two where the first issue is alluded to).

**Response:** We totally understand the reviewers’ concerns about the limitations inherited from case-control study design. According to the reviewer’s suggestion, we have addressed the weaknesses in detail on page 14 second paragraph as follows.

“Also, in the present study, a case-control study was used to access the association of $\gamma$-radiation-induced telomerase activity and GC risk. Therefore, our study definitely has a limitation inherited from this study design, namely that we could not determine whether the observed increased telomerase activity was present prior to cancer development and therefore involved in cancer risk or whether it is a systemic biomarker of gastric cancer because the blood samples were drawn after diagnosis. However, our data showed that there were no significant associations between $\gamma$-radiation-induced telomerase activity and smoking status, drinking status and Hp infection in either GC cases or controls and that the level of $\gamma$-radiation-induced telomerase activity in cases was not significantly associated with the tumor stage and histologic type, suggesting that ability of telomerase activation in PBLs might be more likely to be determined by genetic but not enviromental factors or disease itself and play a role at early stage of GC development. Further confirmation of the cause-and-effect relationship between $\gamma$-radiation-induced telomerase activity and GC development is warranted by using a prospective study design. In addition, the $\gamma$-radiation-induced telomerase activity in PBLs is only a biomarker for the assessment of telomerase inducibility but not clinical relevent event, which might attenuate the biological significance in carcinogenesis. Other biomarkers for risk evaluation in gastric cancer are still needed.”
**Question 3: Page 5 Paragraph 2**
The authors have appropriately explained that they did not collect data on H. pylori infection status and that H. pylori eradication is rarely performed except in cases of confirmed ulcer disease. You cannot however “ignore” the fact that the authors think only a minority of patients received treatment. The authors should merely state that, in their opinion, the influence of H. pylori treatment on the findings of the study should be limited.

**Response:** Based on the reviewer’s suggestion, we have made related revision on Page 5 Paragraph 2 as follows.

“Therefore, in our study, we did not collect the information on the treatment of H. pylori infection in all subjects. We do believe that the influence of H. pylori treatment on the findings of our study should be very limited due to the small percentage”.

**Question 4: Page 8. Power calculations**
Power calculations are done to determine the ability or power (beta) to detect a pre-specified difference in outcomes with a pre-specified significance level (alpha) given the sample size of the study. For most scientific studies alpha (or the threshold of significance) is set at 0.05 and the authors select what they feel is a clinically meaningful difference in a dependent variable. The authors state “the a-priori statistical power of 95.8% was obtained in this study” is inadequate and does not provide the necessary information. I question the validity of the calculations used to obtain the 95.8% figure since this would appear very difficult to obtain given the sample size unless the difference was very large. Also, power is not something that is “obtained”, it is inherent in your study. A proper power statement should read something like “given the sample size of the current study we anticipated __% power to detect a __% change in telomerase activity at the 0.05 significance level”.

**Response:** A previous study indicated that the levels of γ-radiation-induced telomerase activity detected in PBLs were statistically higher in bladder cancer cases than in corresponding controls (1.49 vs. 1.19, p<0.001). Based on these data, we carefully re-evaluated the statistical power of our study using an online tool on website http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html. The parameters were set as follows. Significance level (alpha) was set at 0.05. Sigma value (common SD) was set as 0.9. Finally, our study showed a-priori statistical power of 96%. The power to detect 20% change seem to be higher, possibly due to big sample size. According to the reviewer’s suggestion, we have revised our description to make a proper power statement on Page 8.
Question 5: H. Pylori status

On page 8 paragraph 2 the authors state there were significant differences in H. pylori infection status between cases and controls in addition to differences in smoking and drinking. On page 9 in their multivariate analysis, smoking and drinking were included in the multivariate model but not H. pylori status. Why? The analyses should be repeated adjusting for H. pylori status or the authors should explain why this was not included in the model. Since H. pylori induces chronic inflammation which may alter telomerase activity, this would be a crucial point to address.

Response:
We apologized for the writing mistake. Actually, we have performed the adjustment for the confounding effects of H. pylori infection status in multivariate logistic regression analysis. Related description can be found in “Statistical analysis” of “Materials and Methods” section on Page 8 first Paragraph and prescriptive notes in Table 3 and 5. We missed the words “Hp infection status” on Page 9 when writing the manuscript.
We have added them in revised manuscript and carefully checked the whole paper.

**Minor Revisions**

**Question 1 :** On Page 6 Paragraph 2
The statement that the TRAP-ELISA assay performed comparably to the RT-PCR assay requires a reference supporting this opinion.
**Response:** Supporting reference has been added.

**Question 2 :** On Page 11 Paragraph 3
The p-values and results of previous studies added in parentheses are unnecessary.
**Response:** The description in parentheses has been removed.

**Question 3 :** in Table 1
Since the authors used h.pylori antibody status in their study, “h.pylori infection” should be re-labelled “h.pylori antibody positivity” to more accurately depict this data.
**Response:** Related revisions have been made in manuscript.