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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Author's response to reviews: see over
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Dr. Diana Marshall
Scientific Editor
BioMed Central

Dear Dr. Diana Marshall

Thank you for your letter on 11/25/2009 regarding our manuscript (MS. 2329246262903538). I would also like to thank the reviewers for their time and effort and the positive 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 1:
The report by He et al. is well written, concise, and describes a straightforward series of experiments. The authors report differences in radiation-induced telomerase activity in peripheral lymphocytes in a series of gastric cancer cases and controls. The objectives and hypothesis of the project are relevant clearly stated. The statistical analysis was appropriate and well controlled for differences between cases and controls.

Major Compulsory Revisions:
Question 1. The authors state that their findings support the hypothesis that inherited increased radiation-induced telomerase activity is a risk factor for gastric cancer. I do not think the experiments performed can fully support that hypothesis and needs to be
qualified by expanding on the limitations of the study. First, since blood was drawn in gastric cancer cases after diagnosis we cannot determine whether telomerase activity was increased prior to the development of cancer. In other words, is increased telomerase activity a cause of cancer or the result of a systemic response to malignancy. The latter hypothesis cannot be excluded since increased telomerase activity is seen in activated lymphocytes. Therefore it cannot be determined whether the results of the study are simply a marker of cancer or a risk factor for cancer development.

Response: 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 the cause-and-effect relationship between $\gamma$-radiation-induced telomerase activity and GC development in this retrospective analysis. However, previous studies indicating that telomerase activation was a critical event in human cell immortalization and carcinogenesis have led to the hypothesis that elevated levels of activated telomerase might increase cancer risk by increasing propagation of cells with genomic damage. Further confirmation of this relationship using a prospective study design is warranted.

In revised manuscript, we addressed this issue in more detailed description on Page 14, first paragraph.

**Question 2.** The authors state in their objectives (p4) that they designed this experiment to investigate inherited discrepancy of radiation induced telomerase activity. There is no aspect of the study which addresses whether the observed differences in telomerase activity were inherited or not. Given the issues raised in comment #1, the authors cannot differentiate between inherited differences in telomerase activity and acquired differences in activity either as a result of associated factors (age/smoking/H.pylori) or in response to the development of cancer itself. The role of heritability could be determined by testing telomerase activity in blood-relatives of gastric cancer patients vs controls. Either the authors should provide additional experimental evidence of 'heritability', revise their objectives, or address this weakness in their study.

Response: In a previous study, Kosciolek and Rowley [30] investigated the effect of genetic factors on telomerase activity in PHA-stimulated PBLs and found a heritability of 0.814, indicating that genetic factors played a very critical role in determining the inducibility of telomerase activity. Therefore we hope to expect a similar effect of genetic factors on $\gamma$-radiation-induced telomerase activity in PBLs.
However, unfortunately, the blood samples have not been collected from the relatives of subjects in our study, we could not provide experimental evidence of 'heritability'. Future study is need to confirm our expectation.

We have added more detailed description to address this weakness in our study on Page 12 last paragraph.

Question 3. It would be helpful if the authors provided some indication as to whether they examined the clinical and pathologic characteristics of the malignancies among the cases. Some reports have associated telomerase activity with microsatellite instability while others have noted higher telomerase activity with increasing tumour stage. If telomerase activity did not correlate with clinical stage or tumour characteristics, this would be easy to do and helpful to the readers.

Response: In our study, two hundred and nineteen patients (89%) had adenocarcinoma and 27 patients (11%) had other histological types, including squamous cell carcinoma and undifferentiated carcinoma etc. Twenty-five patients (10%) had stage 1 disease, 54 patients (22%) had stage 2 disease, 103 patients (42%) had stage 3 disease and 64 patients (26%) had stage 4 disease. Our results showed that the level of \( \gamma \)-radiation-induced telomerase activity in cases was not significantly associated with the tumor stage and histologic type, suggesting that telomerase activation might play a role at early stage of GC development.

We have added detailed description on the correlation between telomerase activity and tumour clinical characteristics on Page 4 last paragraph and Page 10 first paragraph.

**Minor Essential Revisions**

**Question.** The authors do not provide any information on a-priori statistical power calculations to determine the magnitude of difference in telomerase activity which could be reasonably detected by their study. This information should be provided.

**Response:** The a-priori statistical power of 95.8% was obtained in the present study. We have added two sentences to state the a-priori statistical power in our study on Page 8 first paragraph.

**Discretionary Revisions**

**Question 1.** The authors used H.pylori antibody to determine H.Pylori status. It is unclear how many cases/controls had active H.pylori infection vs those who had previously treated infection. This information may be useful as active infection may increase telomerase activity in circulating lymphocytes.
**Response:** In China, although H.pylori infection is commonly examined in big hospitals and the positive rate is 40%-70%, most of patients with H.pylori infection do not get any treatment except for the patients with gastric ulcer. Usually, we can ignore the small percentage of treated infection. Therefore, in our study, we did not collect the information on the treatment of H.pylori infection in all subjects. In addition, we did not find any significant associations between $\gamma$-radiation-induced telomerase activity and Hp infection in either GC cases or controls, suggesting that Hp infection might have no modulating effect on $\gamma$-radiation-induced telomerase activity.

We have added detailed description on the treatment information of H.pylori infection on Page 5 last paragraph.

**Question 2:** The authors should comment on the accuracy of their assay compared to more recently developed flourescent real-time PCR based assays.

**Response:** The flourescent real-time PCR based assay (RQ-TRAP) has recently been developed for the measurement of telomerase activity, indicating a highly significant and strong correlation with TRAP-ELISA assay. In comparision with traditional TRAP-ELISA assay, this method is more sensitive and less time-consuming. However, the assay’s coefficient of variation (CV) as an indicator of inter-assay variation was reported to range from 12% to 43% based on different number and type of cells, showing an unstable and not-easy to-control reproducibility. We also found this problem in our preliminary study (data not shown).

We have added a sentence to give a comment on the accuracy of two assays on Page 6 last paragraph.

**Reviewer 2:**
An interesting study examining telomerase activity in controls and GC patients.
Patient selection seemed valid.
Molecular techniques seemed valid.

**Question:** why did you chose lymphocytes instead of tumor cells and normal gastric mucosa?

**Response:** In our study, we aimed to evaluate the inherited inducibility of telomerase activity that could be mainly affected by individual’s genetic variation. Therefore we chose the lymphocyte as a surrogate tissue, but not tumor cells, which could not represent normal genetic background. In addition, lymphocyte rather than normal gastric mucosa is easy to obtain for analysis.
Statistical techniques, and the inferences drawn are questionable.

**Question 1:** The confounding effect of age is difficult to account for, esp since cancer incidence increases with age, and DNA instability may worsen with age.

**Response:** In our study, we found that individuals at least 60 years old had a significantly higher mean level of $\gamma$-radiation-induced telomerase activity than did those younger than 60 years among the GC cases (1.61 ± 1.08 vs. 1.39 ± 0.75; p = 0.060) and healthy controls (1.36 ± 0.76 vs. 1.07 ± 0.60; p <0.001). In addition, we found that higher $\gamma$-radiation-induced telomerase activity is significantly associated with increased risk for GC. These results are consistent with a widely accepted concept that GC incidence increases with age. We hypothesize that, when exposed to the radiation, cells in older people will suffer from more severe DNA damage than those in younger people because DNA repair capacity is getting worse with age. Therefore, the level of telomerase activation as a response to DNA damage will be higher in older people than in younger people. To clearly address the molecular mechanism accounting for the modulating effect of age on $\gamma$-radiation-induced telomerase activity, further investigations are urgently needed.

**Question 2:** although the increase in rad induced telomerase activity is statistically increased, is this really a clinically relevant increase? are there other studies to suggest that this is a CLINICALLY relevant increase in activity? the widely over-lapping values suggest that it may not truly be a relevant activity

**Response:** Previous studies have reported that the telomerase activation is a cellular function in response to endogenous and exogenous mutagen or carcinogen challenge and that telomerase activation was a critical event in human cell immortalization and carcinogenesis. Therefore, in the present study, we used $\gamma$-radiation, which can cause oxidative damage and induce single- or double-strand breaks, to mimic environmental mutagen for the treatment of PBLs and then investigated the association between the inherited inducibility of telomerase activity and GC risk. Actually, 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 increase.

We have added the detailed description on this issue on Page 4 first paragraph.
Question 3: why did you chose the median for high and low activity? this appears to be completely arbitrary? do other studies that are similar in nature make such arbitrary decisions regarding cut-offs? did you do sensitivity analysis to determine if tertiles or quartiles would make more sense? how was this validated?

Response: The radiation-induced telomerase activity is detected as a continual variable in our study. To transform a continual variable to categorical variable for further analysis, commonly, the median value in the controls is arbitrarily chosen as a cutoff to dichotomize subjects into two subgroups. This principle is generally accepted in statistical analysis. In addition, to further validate the finding, we did do the tertile analysis to investigate the dose-response relationship between the level of radiation-induced telomerase activity and GC risk. We found a positive results. We have added several words to give a more detailed description on this issue in our revised manuscript on Page 9 second paragraph.

Question 4: At the end of the results, you state that there was no notable difference in GC risk, but the confidence intervals appear to all be above 1. this does not make sense.

Response: We think that some possible misunderstanding happened. In our results, we aimed to state that there was no notable GC risk difference between older peoples and younger peoples or between never smokers and ever smokers. The value of OR [95%CI] was used to define the level of GC risk for comparison between different subgroups. This comparison is not related with the confidence intervals of any OR value. To avoid any possible misunderstanding, we have made some revision about the sentence structure on Page 10 first paragraph.

Discussion

Question 1: you state that radiation-induced telomerase activity was higher in bladder and lung cancer...were these lymphocytes, or tissue? how much higher? is this in line with your study, or is the difference less for GC?

Response: In previous studies about association between telomerase activity and cancer risk, the radiation-induced telomerase activity was respectively detected in peripheral blood lymphocytes from lung and bladder cancer patients and healthy controls, not in tissues. The radiation-induced telomerase activity (defined as after $\gamma$-radiation telomerase activity/baseline telomerase activity) was significantly higher in bladder cancer cases than that in controls (1.49 vs. 1.19, p<0.001). This result was in line with our results, showing that $\gamma$-radiation-induced telomerase activity was significantly higher in the GC cases than in the controls (1.51 ± 0.93 vs. 1.22 ± 0.66; p
In lung cancer study, the authors used a different definition to measure the relative gamma-radiation-induced telomerase activity, namely the ratio of the net increase of telomerase activity (gamma-radiation induced minus baseline) to the baseline telomerase activity. This value was also significantly higher in lung cancer cases than in corresponding controls (0.730 vs. 0.224, P = 0.0003).

Based on the previous definition in our study and bladder cancer study, the figures could be transformed to 1.730 vs. 1.224 for lung cancer cases and controls, respectively. The difference between cases and controls in lung cancer study is a little bit bigger than those in other two studies.

To address these questions more clearly, in our revised manuscript, we have added more description on Page 11 second paragraph.

**Question 2:** there is no evidence for causality, so some of your conclusions are too strong

**Response:** Because of the limitation inherited from case-control study design, we recognize that we could not determine the cause-and-effect relationship between gamma-radiation-induced telomerase activity and GC development in this retrospective analysis. Further confirmation of this relationship using a prospective study design is warranted. To rule out any possibility of misleading, we attenuated our conclusions based on the reviewer’s suggestion by making corresponding revision on Page 14 second paragraph.