EMBO
reports

Telomeric epigenetic response mediated by Gadd45a regulates stem cell aging and lifespan

Daojun Diao, Hu Wang, Tangliang Li, Zhencan Shi, Xiaoqing Jin, Tobias Sperka, Xudong Zhu, Meimei Zhang, Fan Yang, Yusheng Cong, Li Shen, Qimin Zhan, Jing Yan, Zhangfa Song and Zhenyu Ju

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 19th Dec 17

Thank you for the submission of your research manuscript to EMBO reports. We have now received reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, all three referees highlight the general interest of the findings. However, all three referees have raised a number of concerns and suggestions to improve the manuscript, or to strengthen the data and the conclusions drawn. As the reports are below, I will not detail them here. Most importantly though, we want to draw your attention the major concerns of referee #2, in particular his/her point on the use of ChIP-PCR to detect and quantify alterations in telomeric chromatin, which we think needs to be addressed as indicated by the referee. Also an experiment knocking down a BER protein (e.g. APE-1) to confirm the role of BER in the process, would be required, as all three referees have concerns regarding the use of the pharmacological inhibitor.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript and in a point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

REFEREE COMMENTS

Referee #1:

This study examines the role of Gadd45a in the DNA damage response (DDR) signaling at critically short telomeres. Using the G3Terc-/- mouse model harboring critically short telomeres, the authors show that knocking out Gadd45a improves intestinal stem cell function. They provide evidence that the mechanism is related to Gadd45a promoting chromatin structure at subtelomeric and telomeric regions that facilitate DDR signaling. Use of a BER inhibitor suggests that Gadd45a promotes DNA demethylation in cooperation with the BER machinery. The study is well done, and the data support the conclusions for the most part. However, there are a few issues that need to be addressed.

Major comments.

1. Figure 1 B and C. Gadd45a might be enriched at telomeres in G3Terc-/- cells compared to wild type because the overall protein levels are higher in the mutant mice (Fig. 1A). Is the enrichment specific for telomeres, or is enrichment also observed throughout the genomes (i.e. also at ALU repeats).

2. The CRT0044876 inhibitor needs to be better described and citations are needed. This is an inhibitor of APE1 enzyme, and a positive control should be used to ensure the inhibitor is working to inhibit BER as expected in these cells and in mice. For example, an increase in abasic sites should be observed (see Madhusudan et al, NAR, 2005). Kits are available for abasic site detection.

3. Figure 5D. Demonstrating that the gammaH2AX foci colocalize with telomeric DNA (TIFs; markers of telomere dysfunction induced foci) would greatly strengthen the conclusion that "Gadd45a-mediated BER" facilitates the DDR signaling at short telomeres. The TIF assay is standard in the telomere field.

4. Gemcitabine is not a specific NER inhibitor (i.e. it does not only inhibit NER). It is a chain terminator that gets incorporated into DNA during DNA synthesis and DNA replication. A lack of rescue with gemcitabine, therefore, is difficult to interpret. I suggest removing the conclusion that inhibition of NER does not "alleviate DDR" from the text and the abstract. The data suggesting NER is not involved is too weak.

5. Figure 6. It is inaccurate to state that BER inhibition or GADD45A knockdown "prevents" or "rescues" replicative senescence. Rather these factors appear to delay senescence, but the population doubling appears to plateau in the knock down cells at passage 8 (Fig. 6 B). Unless these cells have been immortalized with telomerase they should eventually senesce or undergo apoptosis as the telomeres cannot be maintained.

6. Figure 6E and F. Do the colon samples from old individuals versus young also show more gammaH2AX foci or TIFs? In other words, does the higher GADD45A expression in the tissue from older individuals have any functional consequence? A description of the source of these colon samples is needed (i.e. average age and range of ages, and gender).

Minor comments
1. Numerous grammatical errors require correction
2. Page 8, please correct "bona fade" to "bona fide".

Referee #2:

This manuscript describes a novel role of the GADD45a, an important regulator of DNA de-methylation, at critically short telomeres. The authors report that the depletion of GADD45a alleviates some of the phenotypes associated with telomerase deficiency. They provide evidence that this is due to GADD45a's role as an adaptor facilitating the dual activities of DNA demethylation and base excisison repair. An inhibitor of the latter mimics the effects of GADD45a depletion

Overall, I found elements of this manuscript very appealing and of significant interest. The potential synergism between epigenetic and BER regulation is particularly novel - with respect to telomeres.

Here are my major concerns:

1. The critical negative issue with this work stem from the use of ChIP-PCR to detect and quantify alterations in telomeric chromatin. This is not the appropriate method to use due to the repetitive nature of the TTAGGG sequence. The widely excepted practice is to conduct ChIP followed by dot blot and southern blot using radio-labelled probes (See all work by de Lange, Karlseder, Chang, Blasco etc). Also related to the ChIP assays - the data in the column graphs is presented as "percentage of telomeric DNA levels". I wonder if this accurate, is the input DNA not diluted? If not, the data suggests that ~75% of telomeric chromatin has H3K9me3 (see Figure 4C), which is amazing. Can the authors please clarify the method of quantification?

For this reviewer, this is the critical issue that prevented me from giving a thoroughly positive review. It can be corrected or retested such that it is in-line with norms of the field.

2. The results of the experiments using the APE-1 inhibitor (they should just call it that instead of CRT.....). However, though it may be normal to use 100uM, this dose is extremely high. I would like to see the effects of lower doses to see if there is a true pharmacokinetic response. They should also include a positive control to show that the inhibitor is functioning as proposed in their hands.

In addition, though I am aware of different modes of action, I think it would really bolster the outcomes of these experiments if the authors could obtain a similar effect by knocking down APE-1 or a related BER protein. This would confirm the important link between GADD45a and BER.

Other minor comments include:

1. The authors should proof read the paper and correct grammatical errors. They overuse "the" as a prefix. For example " Mechanistically, Gadd45a facilitates the telomeric heterochromatin". In other cases, it is absent. For example "attrition of telomere triggers the DNA damage response".
2. I might be wrong but I did not see any genotyping in this manuscript. It would be appropriate to include the essential genotyping data.
3. I don't understand the reasoning for looking into anaphase bridges as a read-out of telomere uncapping. Anaphase bridges can also arise from replicative complications or prolonged mitosis. Is there any evidence that these cells display "telomere uncapping"? The authors should re-consider their reasoning and amend this section with necessary citations.
4. Page numbers should be added to help reviewers!

Referee #3:

In this manuscript, the authors report a role for GADD45a in the cellular response to telomere dysfunction. The first part of the manuscript is descriptive and shows that depletion of GADD45a alleviates several of the phenotypes seen in the third generation (G3) telomerase knockout mice (Terc-/-). In particular, the authors focus on the intestinal stem cells maintenance defects triggered by telomere erosion. The authors show that depletion of GADD45a in the context of telomerase deficiency rescue intestinal stem cell defects, body weight loss and extends lifespan. This finding is novel and suggests that GADD45a is critical for the response to telomere dysfunction. This portion of the manuscript is well performed, well controlled and of high standards.

The second portion of the manuscript aims to define what is the role of GADD45a in response to telomere dysfunction. The authors conclude that upon telomere attrition GADD45a triggers localized DNA demethylation at chromosome ends a process that somehow would enhance the DNA damage response triggered by telomere dysfunction. Moreover, the author report that inhibition of the Base Excision Repair (BER) pathway rescues some of the phenotypes induced by telomere dysfunction, in a manner that is similar to that observed upon GADD45a depletion.

This manuscript has the potential of revealing a new role for GADD45a and the BER pathway in

response to telomere dysfunction. As such, this work would be of interest to the field of telomere biology, DNA damage, and aging. However, the complete lack of mechanistic insight into the function of GADD45a and the BER pathway in this process limits the interest and relevance of this work.

Also, I have the following major concerns:

-Previous work showed that GADD45a plays a role in p53 stabilization upon DNA damage induction (Jin S et al. Oncogene 2003). This could explain several of the findings reported in this manuscript, suggesting that GADD45a depletion would act by dampening p53-mediated DNA damage response). Remarkably, the authors fail to cite this paper and decided not test whether in the absence of GADD45a p53 induction is compromised.

-Regarding the methylation status (figure 4) these experiments lack an essential control: the methylation status of other non0telomeric repetitive elements. This is required to define whether GADD45a acts specifically at telomeres in response to telomere erosion.

-The authors suggest that the lack of demethylation in the response of telomere erosion explains why in the absence of GADD45a cells with critically short telomeres do not induce a DNA damage response. However, an alternative explanation is that in the absence of GADD45a the lack of a strong DNA damage response suppresses DNA demethylation. Experiments aimed at addressing whether depletion of other DNA damage response factors de-methylation occurs would address this critical point.

-A critical role of BER in response to telomere erosion is unexpected and requires further confirmation. The data obtained using chemical inhibition would be significantly strengthened using genetic approaches (e.g., shRNA in fibroblasts reaching replicative senescence).

-The connection between GADD45a and the BER pathway upon telomere erosion is unclear to this reviewer. The authors should test whether BER inhibition in G3 dKO cells has any effect. This experiment could potentially provide evidence that these factors act in the same pathway.

1st Revision - authors' response | 17th May 18

Dear referees:

We sincerely thank all reviewers for their strong interests on our findings and further the reviewers raised valuable suggestions on improving the quality of our data. Based on these suggestions, we conducted new experiments and extensively revised our manuscript. The corresponding changes in the manuscript are highlighted with blue texts.

Furthermore, the reviewer comments are laid out below. Our response is given in bold font.

Referee #1:

Major comments.

1. Figure 1 B and C. Gadd45a might be enriched at telomeres in G3Terc-/- cells compared to wild type because the overall protein levels are higher in the mutant mice (Fig. 1A). Is the enrichment specific for telomeres, or is enrichment also observed throughout the genomes (i.e. also at ALU repeats).

Response: The point raised by the reviewer is valid. In our analysis， we found that G3Terc-/- cells showed higher expression of Gadd45a (Fig. 1A). Since that DNA damages are only occurred at telomeres in cells from G3Terc-/-, we proposed that Gadd45a is enriched at telomere, other than throughout the whole genome. To investigate whether the Gadd45a protein is specifically enriched on the telomere other than other repetitive sequence in the mouse genome, we conducted ChIP-PCR assay and found that Gadd45a protein at ALU repeats was not increased in cells from G3Terc-/- mice (revised Fig. 1C). This data further

indicates that a local specific role of Gadd45a.

2. The CRT0044876 inhibitor needs to be better described and citations are needed. This is an inhibitor of APE1 enzyme, and a positive control should be used to ensure the inhibitor is working to inhibit BER as expected in these cells and in mice. For example, an increase in abasic sites should be observed (see Madhusudan et al, NAR, 2005). Kits are available for abasic site detection.

Response: We thank the reviewer for this suggestion, which will strengthen our data on Gadd45a-BER at telomeres in G3 Terc-/- mice. CRT0044876 is an inhibitor of APE1 enzyme. For the positive control, we used the kit (Dojindo Molecular technologies, Inc.) and conducted AP site accumulation assay to investigate the enzyme activity of APE1 after APE1 inhibitor CRT0044876 treatment. We found that APE1 enzyme activity are all inhibited in organoid culture, MEF cells and mice crypts, as indicated by more abasic site (see Appendix Fig. S9G). These data suggest that CRT0044876 is working to inhibit BER as expected in both cells and in mice. Furthermore, as suggested by the reviewer, we have include a reference on this inhibitor and revised the corresponding text to have a clear description on CRT0044876 inhibitor.

3. Figure 5D. Demonstrating that the gammaH2AX foci colocalize with telomeric DNA (TIFs; markers of telomere dysfunction induced foci) would greatly strengthen the conclusion that "Gadd45a-mediated BER" facilitates the DDR signaling at short telomeres. The TIF assay is standard in the telomere field.

Response: The reviewer's point is valid. TIF assay is the "golden standard assay" in telomere field. In revised Figure 5D, we conducted TIF assay. TRF2 staining was used as the marker for telomeres, while gammaH2AX antibody staining was used to shown the DSBs on telomeres. We show that a strong co-localization between TRF2 and gammaH2AX in G3Terc-/- cells compared to the wild type cells.

4. Gemcitabine is not a specific NER inhibitor (i.e. it does not only inhibit NER). It is a chain terminator that gets incorporated into DNA during DNA synthesis and DNA replication. A lack of rescue with gemcitabine, therefore, is difficult to interpret. I suggest removing the conclusion that inhibition of NER does not "alleviate DDR" from the text and the abstract. The data suggesting NER is not involved is too weak.

Response: We agreed with the reviewer that Gemcitabine is not a specific NER inhibitor. Therefore, we could not rule out the possibility that failure to rescue G3 Terc-/- by Gemcitabine is caused by the strong deleterious effects on Gemcitabine in DNA relocation. Thus, we have removed the data about gemcitabine and NER inhibitor and revised the manuscript accordingly.

5. Figure 6. It is inaccurate to state that BER inhibition or GADD45A knockdown "prevents" or "rescues" replicative senescence. Rather these factors appear to delay senescence, but the population doubling appears to plateau in the knock down cells at passage 8 (Fig. 6 B). Unless these cells have been immortalized with telomerase they should eventually senesce or undergo apoptosis as the telomeres cannot be maintained.

Response: The point raised by the reviewer is valid. After careful reading the data and manuscript, we agree with the reviewer that BER inhibition or GADD45A knockdown only delay the onset of replicative senescence. Therefore, we changed the manuscript accordingly and the title of Figure 6.

6. Figure 6E and F. Do the colon samples from old individuals versus young also show more gammaH2AX foci or TIFs? In other words, does the higher GADD45A expression in the tissue from older individuals have any functional consequence? A description of the source of these colon samples is needed (i.e. average age and range of ages, and gender).

Response: Thanks the reviewer for these thoughtful suggestion. In order to solve these concerns, we conducted gammaH2AX antibody staining in colon samples from young and old

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people. The results showed that the frequency of gammaH2AX positive crypts is increased in old human colons (Figure 6E). A description of the sources of colon samples used in our analysis were summarized in Appendix table 1.

Minor comments
1. Numerous grammatical errors require correction

Response: We are sorry for the dramatic errors in previous version of our manuscript. In the revised manuscript, we have carefully read the manuscript, and correct all possible grammatical error.

2. Page 8, please correct "bona fade" to "bona fide".

Response: Thank you very much for the careful reading, we have corrected the "bona fade" into "bona fide". Furthermore, we have proof-read the text and correct all typos.

Referee #2:

Here are my major concerns:

1. The critical negative issue with this work stem from the use of ChIP-PCR to detect and quantify alterations in telomeric chromatin. This is not the appropriate method to use due to the repetitive nature of the TTAGGG sequence. The widely excepted practice is to conduct ChIP followed by dot blot and southern blot using radio-labelled probes (See all work by de Lange, Karlseder, Chang, Blasco etc). Also related to the ChIP assays - the data in the column graphs is presented as "percentage of telomeric DNA levels". I wonder if this accurate, is the input DNA not diluted? If not, the data suggests that ~75% of telomeric chromatin has H3K9me3 (see Figure 4C), which is amazing. Can the authors please clarify the method of quantification?

Response: We agree with reviewer that, due to repetitive nature of telomere region, ChIP-PCR may not be ideal for quantification process. Thus, we followed the suggestions and dot-blot with ChIPed telomeric chromatins. The new results showed that the tri-methylation of H3K9 and HP1a level were reduced at telomere of G3Terc-/- mice, which were increased in G3-dKO mice. In contrast, the acetylation of H3K9 was increased at telomere of G3Terc-/- mice, which was reduced in G3-dKO mice (see new Fig. 4C-E and Fig. 5F-H). These data indicates that the telomeric chromatin is relaxed in G3Terc-/- mice, which is condensed in G3-dKO mice. In addition, for the ChIP-qPCR quantification, the input DNA was diluted for the qPCR analysis. Therefore, the value of ~75% is the relative ratio of qPCR value from the antibody-ChIP produce to that from the input, which does not indicate that ~75% telomeric DNA has H3K9me3. To avoid this confusion, we have change this in the revised Figures.

2. The results of the experiments using the APE-1 inhibitor (they should just call it that instead of CRT.....). However, though it may be normal to use 100uM, this dose is extremely high. I would like to see the effects of lower doses to see if there is a true pharmacokinetic response. They should also include a positive control to show that the inhibitor is functioning as proposed in their hands.

Response: The concerns raised by the reviewer is valid. Initially, we chose to use the concentration (100um) following the method as described in paper (Wilson DMr, Simeonov A. Small molecule inhibitors of DNA repair nuclease activities of APE1. Cell Mol Life Sci 2010). In the revised manuscript, we followed the reviewer's suggestion and optimize the drug treatment by testing a serial of CRT0044876 concentrations (1, 5, 10μM)(Madhusudan et al, NAR, 2005). We found that at the concentration of 10μM, the APE1 inhibitor CRT0044876 gave better rescue effects in G3Terc-/- cells than 100uM of CRT0044876 (see Figure 5). For a positive control of APE1 inhibitor CRT0044876, AP site accumulation assay was conducted and the inhibition efficiency of APE1 inhibitor (CRT0044876) was shown in (see Appendix Fig. S9G).

In addition, though I am aware of different modes of action, I think it would really bolster the outcomes of these experiments if the authors could obtain a similar effect by knocking down APE-1 or a related BER protein. This would confirm the important link between GADD45a and BER.

Response: We appreciate the reviewer's suggestion to use the genetic approaches to assay the rescue effects of APE1 inhibition. Therefore, we knocked down APE1 in G3Terc-/- cells and checked the methylation status of sub-telomeric region. The results showed that the methylation level was reduced in sub-telomeric region of G3Terc-/- mice, which was increased by APE1 knockdown (see Appendix Fig. S10). Furthermore, we knocked down APE1 in human FB (WI-38), the β-gal staining showed that knockdown of APE1 could significantly delay cellular senescence (see Appendix Figure S11).

Other minor comments include:

1. The authors should proof read the paper and correct grammatical errors. They overuse "the" as a prefix. For example " Mechanistically, Gadd45a facilitates the telomeric heterochromatin". In other cases, it is absent. For example "attrition of telomere triggers the DNA damage response".

Response: We have carefully proof-read the manuscript, and corrected the prefix of "the" and some grammatical errors.

2. I might be wrong but I did not see any genotyping in this manuscript. It would be appropriate to include the essential genotyping data.

Response: We thank the reviewer for this reminding. We have added the genotyping information in Appendix Fig. S1A.

3. I don't understand the reasoning for looking into anaphase bridges as a read-out of telomere uncapping. Anaphase bridges can also arise from replicative complications or prolonged mitosis. Is there any evidence that these cells display "telomere uncapping"? The authors should re-consider their reasoning and amend this section with necessary citations.

Response: The point raised by the reviewer is valid, and in anaphase bridges could also arise from replicative complications or prolonged mitosis. Anaphase bridge is a very often happened cytogenetic phenomenon in cells from G3Terc-/- mice. In G3Terc-/- cells, the short telomeres easily induce telomere-telomere fusion, which subsequently generating anaphase bridge. Therefore, anaphase bridge is often used a marker for the telomere dysfunction induced DNA damage response. Nevertheless, anaphase bridge does not necessarily mean "telomere uncapping", therefore, we have amend this section by changing "telomere uncapping" to "telomere dysfunction".

4. Page numbers should be added to help reviewers!

Response: According to the reviewer's suggestion, we inserted page numbers in the revised manuscript.

Referee #3:

Also, I have the following major concerns:

1. Previous work showed that GADD45a plays a role in p53 stabilization upon DNA damage induction (Jin S et al. Oncogene 2003). This could explain several of the findings reported in this manuscript, suggesting that GADD45a depletion would act by dampening p53-mediated DNA damage response). Remarkably, the authors fail to cite this paper and decided not test whether in the absence of GADD45a p53 induction is compromised.

Response: We appreciate the reviewer's comment and have added the citation of paper (Jin S et al. Oncogene 2003). According to this paper, GADD45a function as stabilizer of p53 upon

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DDR. In our G3Terc-/- mice, the protein level of Gadd45a and p53 are both increased (Fig. 3A and B), therefore it is possible that GADD45a functions as p53 stabilizer. Apart from this, our data suggest that GADD45a also function at the upstream of p53 according to the fact that the upstream regulator of p53 (e.g. 53BP1 and pATM) was activated in G3Tec-/- mice but reduced in G3-dKO mice (see Fig. 3E and F; Appendix Fig. S4E and F). Therefore, it is plausible that GADD45a deletion affects both the p53 stabilization and the DDR induction at dysfunctional telomeres. We have modified the manuscript accordingly.

2. Regarding the methylation status (figure 4) these experiments lack an essential control: the methylation status of other non0telomeric repetitive elements. This is required to define whether GADD45a acts specifically at telomeres in response to telomere erosion.

Response: We are grateful that the reviewer point out the control experiments of chromatin methylation assay. In the revised manuscript we characterized other well-document repetitive elements in mouse genome and found that there was no change of methylation status in the cells from all the genotypes (see Appendix Fig. S6B). Our data strongly suggested that Gadd45a regulates the DNA methylation specially at sub-telomeric regions.

3. The authors suggest that the lack of demethylation in the response of telomere erosion explains why in the absence of GADD45a cells with critically short telomeres do not induce a DNA damage response. However, an alternative explanation is that in the absence of GADD45a the lack of a strong DNA damage response suppresses DNA demethylation. Experiments aimed at addressing whether depletion of other DNA damage response factors de-methylation occurs would address this critical point.

Response: We thank the reviewer for the comment that loss of Gadd45a could suppress the DDR and indirectly dampen the DNA demethylation. However, we do feel that Gadd45a-BER could directly involve in the telomere/Sub-T chromatin status maintenance, since chemically and genetically modulate the BER pathway could generate similar chromatin phenotype in G3 Terc-/- as shown in Gadd45-G3 dko cells. To further substantiate our data, we deleted Gadd45a independent DDR response gene (p21) by siRNA, since p21 has been link to the intestinal stem cell defects in G3 mice and loss of p21 could partially rescues the G3Terc-/- mice. The results showed that knockdown of p21 did not change the methylation status of telomeric region in G3Terc-/- mice (Appendix Fig. S7). Therefore, our data indicates that Gadd45a could specially regulate the cell homeostasis by regulating the chromatin at the telomere.

4. A critical role of BER in response to telomere erosion is unexpected and requires further confirmation. The data obtained using chemical inhibition would be significantly strengthened using genetic approaches (e.g., shRNA in fibroblasts reaching replicative senescence).

Response: We thank the reviewer for this suggestion. This point also was raised by reviewer 2. To genetically investigate the BER in G3 Terc-/- cells, we knocked down APE1 in G3Terc-/- MEF cells and human FB (WI-38) by using siRNA. The sub-telomeric DNA methylation level was increased in G3Terc-/- MEF cells after APE1 knockdown (see Appendix Fig. S10). In human FB (WI-38), the β-gal staining showed that knockdown of APE1 could significantly delay replicative senescence (see Appendix Fig. S11).

5. The connection between GADD45a and the BER pathway upon telomere erosion is unclear to this reviewer. The authors should test whether BER inhibition in G3 dKO cells has any effect. This experiment could potentially provide evidence that these factors act in the same pathway.

Response: The point raised by the reviewer is valid. This point is related to #4 point by the reviewer. In order to solve the concerns, we investigate the epigenetic effects of APE1 inhibition in G3-dKO organoid culture. The results showed that APE1 inhibition did not change the organoid growth of G3-dKO mice anymore (see Appendix Fig. S9A and B), these support our interpretation that Gadd45a function through the BER pathway in G3Terc-/- mice.

2nd Editorial Decision 7th Jun 18

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the two referees that were asked to re-evaluate your study (you will find enclosed below). Referee #1 was not able to look at the revised manuscript, but going through your point-by-point response, we consider his/her points as adequately addressed.

As you will see, the remaining two referees now support the publication of your manuscript in EMBO reports. However, they raised some final concerns and/or suggestions, we ask you to address in a final revised manuscript.

Further, I have the following editorial requests:

- Please add the running title to the manuscript title page.

- The abstract is currently too long. Please shorten the abstract to not more than 175 words!

- Please remove the figure legends from the image files, and include these into the main manuscript text file in a section called 'Figure Legends'.

- Your manuscript has currently 6 main figures, and 11 Appendix figures. As already mentioned in my previous decision letter, we now prefer to present important supplementary data in the Expanded View format (which will be displayed in the main HTML of the paper in a collapsible format). You can select up to 5 images from your Appendix as Expanded View, which we suggest to do. Please follow the nomenclature Figure EV1, Figure EV2 etc. in the manuscript text and the legends. The figure legend for these should be included in the main manuscript document file in a section called 'Expanded View Figure Legends' after the main 'Figure Legends section'. The additional Supplementary material you can then keep in the Appendix. Please provide the Appendix as one single pdf labelled Appendix. The Appendix includes a table of content on the first page, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text, the Appendix TOC, their legends and the labels (the S is currently missing). For more details please refer to our guide to authors:
http://embor.embopress.org/authorguide#manuscriptpreparation

- Please add a conflict of interest statement, and author contributions to the manuscript text (before the acknowledgements).

- Please deposit the RNA-seq. data (transcriptome analysis) at a public data repository (e.g. the Gene Expression Omnibus), and provide the accession number in the methods section (in a section called 'Data Availability' - see also section F of the author checklist).

- Please provide an ORCID for the co-corresponding author (Diao), and link this to his EMBO reports profile.
We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

REFEREE COMMENTS

Referee #2:

The authors have significantly improved on the initial submission and added important controls to validate their initial findings.

I have two concerns; 1 major and 1 minor.

I believe that it is necessary to perform the dot-blot ChIP showing enrichment of Gadd45 at telomeres, as is implied in Figure 1C. The authors show that they can do the assay elsewhere so, for consistency, this should be done.

A minor issue is the terminology used with respect to chromatin modifications. To the best of my knowledge, it is not commonplace to use the terms "chromatin relaxation", "relaxed chromatin" or "to relax chromatin". Terms such as accessible, euchromatic, condensed, decondensed, open vs closed configurations are typically used. I also find some of the section titles to be overly complex. For instance, "Gadd45a, integrated into BER machinery, functions as a site specific chromatin relaxer in response to telomere dysfunction" and "Targeting epigenetic modulating factor to combat stem cell aging". These lack clarity and should be amended.

Referee #3:

In this revised manuscript the authors were responsive to the reviewer comments addressing several of the points raised. Overall this manuscript reports on interesting observations regarding the role of GADD45a and the BER pathway following telomere dysfunction and the novel data strengthens the data.

I still have two points that the reviewers should address:

The new experiments using knockdown of APE1 are not convincing. Supplemental figure 10 reports the level of methylation at subtelomeres, and it appears that the effect of the control siRNA is higher than the impact of siAPE1. Similarly, Supplemental figure 11 which reports the effect of siAPE1 on senescence in fibroblasts is not convincing. Growth curves and other parameters should be included to show that APE1 knockdown results in diminished senescence following telomere dysfunction.

Quantification of CHIP experiments: The authors cannot use arbitrary units to quantify the % of telomeres recovered following ChIP, this is not acceptable.

2nd Revision - authors' response 26th Jul 18

Dear referees:

We sincerely appreciate all reviewers for the consideration on our revised manuscript and for the valuable questions and suggestions. Based on these, we conducted new experiments and also extensively revised our manuscript. The corresponding changes in the manuscript are highlighted with blue texts.

Furthermore, the reviewer comments are laid out below. Our response is given in bold font.

Referee #2:

Major concern: I believe that it is necessary to perform the dot-blot ChIP showing enrichment of Gadd45 at telomeres, as is implied in Figure 1C. The authors show that they can do the assay elsewhere so, for consistency, this should be done.

Response: Thanks the reviewer for these thoughtful suggestion. We conducted the dot-blot assay following Gadd45a-ChIP, the results showed that Gadd45a is enriched at telomere (revised Fig 1D), which is consistent with ChIP-qPCR results (Fig 1C).

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Minor concern: A minor issue is the terminology used with respect to chromatin modifications. To the best of my knowledge, it is not commonplace to use the terms "chromatin relaxation", "relaxed chromatin" or "to relax chromatin". Terms such as accessible, euchromatic, condensed, decondensed, open vs closed configurations are typically used. I also find some of the section titles to be overly complex. For instance, "Gadd45a, integrated into BER machinery, functions as a site specific chromatin relaxer in response to telomere dysfunction" and "Targeting epigenetic modulating factor to combat stem cell aging". These lack clarity and should be amended.

Response: We have carefully re-proof read the manuscript, and corrected the terminology used with respect to chromatin modifications. We have also revised and simplified the section titles and figure titles.

Referee #3:

1. The new experiments using knockdown of APE1 are not convincing. Supplemental figure 10 reports the level of methylation at subtelomeres, and it appears that the effect of the control siRNA is higher than the impact of siAPE1.

Response: The percentage of unmethylated CpGs in Supplemental figure 10A (revised Fig EV 4A) was quantified in the revised Fig EV 4B. The level of unmethylated CpGs was increased in G3Terc-/-(si-Ctrl) in comparison to WT/si-Ctrl, and significantly reduced after si-APE1 treatment (G3Terc-/-(si-APE1)). Supplemental figure 10B (revised Fig EV 4C) showed the knockdown efficiency of si-APE1 in G3Terc-/- mice cells.

2. Similarly, Supplemental figure 11 which reports the effect of siAPE1 on senescence in fibroblasts is not convincing. Growth curves and other parameters should be included to show that APE1 knockdown results in diminished senescence following telomere dysfunction.

Response: The point raised by the reviewer is valid. According to the reviewer's suggestions, we conducted new experiments and the growth curves showed that the human fibroblast grew faster than in control after si-APE1 treatment (Fig EV5F). The expression of senescence related genes was also reduced after si-APE1 treatment (Fig EV5G). These data indicated that APE1 knockdown could delay senescence in human fibroblast.

3. Quantification of CHIP experiments: The authors cannot use arbitrary units to quantify the % of telomeres recovered following ChIP, this is not acceptable.

Response: The point raised by the reviewer is valid. For the quantification of CHIP experiments, we changed the label to "Relative DNA signal to input (%)".

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Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ➔ (Do not worry if you cannot see all your text once you press return)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The number of animals used for study is above 4.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For animal studies, the number of animals is above 4.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For the analysis, there was no exclusion.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NO
For animal studies, include a statement about randomization even if no randomization was used.	The statistic of sample is absolute random.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	For the animal studies, the animals were chosen according to age and body weight match.
5. For every figure, are statistical tests justified as appropriate?	yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA
Is there an estimate of variation within each group of data?	error bar
Is the variance similar between the groups that are being statistically compared?	yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	anti-γH2AX (Millipore, 05-636,1:200)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	The animal breeding and experiments were conducted at the animal facility Jinan University with the approval of the Animal Care and Ethics Committee.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	ok

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
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F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	ok
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20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
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G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA