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Oxidative stress induces chromosomal instability through replication stress in fibroblasts from aged mice

Guan Chen, Zhenhua Li, Kenji Iemura and Kozo Tanaka
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MS TITLE: Oxidative stress induces chromosomal instability through replication stress in fibroblasts from aged mice

AUTHORS: Guan Chen, Zhenhua Li, Kenji Iemura, and Kozo Tanaka
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We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The manuscript by Chen and colleagues investigates the relationship between chromosome mis segregation, oxidative stress and aging. By using fibroblast model isolated from young and aged

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mice, the authors investigate how aging affects the fidelity of chromosome segregation and micronucleation via external and internal oxidative stress. The authors conclude that fibroblasts extracted from old mice exhibit elevated ROS levels and a decline of mitochondrial functions, pointing to an increase in oxidative stress. The authors also find a higher frequency of lagging chromosomes and micronuclei in old fibroblast and they show that treating cells with antioxidants reduces chromosome mis segregation. Finally, the authors report a correlation between aging and appearance of 53BP1 nuclear bodies and phosphorylated ATM protein, concomitant with a decrease in lagging chromosome after supplementing cells with nucleotides. Based on these data they conclude that oxidative stress induces replication stress and chromosome mis-segregation in old mouse fibroblasts.

Overall the experimental part of this work is well executed and results are presented in a coherent and clear manner. The study is relevant for both the fields of aging and chromosomal instability. There are, however, some concerns over the novelty of the study. Chromosome mis segregation, oxidative stress and aging in mouse fibroblasts have never been studied in the same model system (fibroblasts), but several of these links have studied separately in the past. It is known that oxidative stress causes chromosome mis segregation in drosophila oocytes (Perkins et al 2016) and human-hamster hybrid cells (Limoli & Giedzinski 2003). Moreover, oxidative stress has been been linked to aging in mice (Forster et al 1996, Navarro et al 2002) and rat (Gilmer et al 2010) brains. The authors should at minimum better discuss how their study advances the field, and cite those papers in their discussion. In addition, there are a number of experimental concerns that the authors should address before publication.

Patrick Meraldi

Comments for the author

Major points:
1. Throughout the authors refer to chromosomal instability in aging cells, but they never actually check for presence of aneuploidy in their model, and instead rely on indirect readouts such a lagging chrosomes and micronucleis. While those can lead to chromosome gain/loss it is not automatic. Since the authors report 6% of chromosome missegregation in aged cells (Figure 1C) one would expect to see an increase of aneuploidy in their model. Therefore the authors should compare the karyotypes of old and young fibroblasts.

2. All the rescue experiments presented in Figure 4 (oxidative stress), Figure 5 (replication stress) and Figure 6 (oxidative stress and stabilized microtubules) show partial and sometimes weak rescues. Altough the authors present their results in carefuly manner, their discussion nevertheless implies a causal chain with aging on top driving oxidative stress causing replication stress which in turns leads to stabilized microtubules. One caveat is that in their model the authors did not directly test whether replication stress is upstream of stabilized microtubules. Second, given that the observed effects are only partial, the authors should better discuss these partial rescues, or test whether alternative treatments yield clearer results. Would a combined NAC and Mito Q treatment give a better rescue? Would a direct inhibition of the DNA damage pathway, such as inhibition of ATR show a better rescue. Also, in terms of additive effect, would a combination of NAC and UMK57 give a non-additive effect, as implied by their model? Some of these experiments could put this study on a firmer footing, by providing additional controls and a certain redundancy.

Minor points :
1- The images presented in Figure 2A have an insufficient quality, the manuscript would benefit from better examples.
2- Figure 3C would benefit from splitting the green and red channel in the presented images to better visualize the reduction in the red channel in old fibroblasts. It also would be useful to explain the meaning of red and green signal in the legend of the figure.

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Reviewer 2

Advance summary and potential significance to field

Chen et al. suggest a link between oxidative stress and chromosome instability (CIN) in aging. They show that CIN emerges in the form of abnormal nuclear structures or defective mitoses in ex vivo cultures of primary fibroblasts from aged mice, but not from young mice. They furthermore propose that replication stress, a hallmark consequence of aging, is induced by oxidative stress and together with microtubule stabilization are the mechanisms that underlie CIN in aging.

In terms of the overall significance, this paper does a nice job of connecting a series of Â“dotsÂ”, but each pair of dots have already been connected in prior work. Although the overall conclusions are mainly confirmatory, in balance, we feel that the paper makes a valuable contribution. We particularly appreciate the experiments where cultures are grown in physiological normoxia. The authors show that oxidative stress and mitochondrial dysfunction induce replication stress and CIN in primary cultures of aged fibroblasts, as expected from prior work. However, since aneuploidy - which is a consequence of CIN - is a hallmark feature of aging (eg. Naylor and Deursen, 2016) and CIN has been suggested to be observed in models of aging (eg. see a review doi: 10.1002/mc.22539), the novelty of this work is limited to the linkage between oxidative stress, replication stress and CIN in aged fibroblasts in one experimental setting.

The methods, as well as the results, are clearly presented. The introduction should be improved by a better discussion of prior literature on CIN in aging and the novel points should be clearly stated. For example, the authors state that work on mosaic variegated aneuploidy syndrome is the only prior and indirect evidence of CIN in aging. Prior literature on aneuploidy in aging which is predictive for the underlying CIN should also be mentioned, as discussed above. In addition, the authors should clarify specific interpretations of their results (see specific points below).

Below, we detail more experimental work and further analysis that is needed to strengthen the main results. With the additional experiments, analysis and clarifications in the text as asked, this manuscript will be a good fit for Journal of Cell Science and provide important insight in the fields of chromosomal instability and aging.

Major comments:

- the authors should assess the possibility that the effects in aged fibroblasts (eg increased MN) reflect the presence of tetraploid cells in the population (a common occurrence in cultures of primary mouse cells). Especially since Fig. S1b implies that this population is large (around 15-20%). All the comparisons between old and new fibroblasts should be restricted to near-diploid cells.

- the type of CIN observed in aged fibroblasts should be further examined, which would help clarify the underlying mechanism. The authors propose that both replication stress and microtubule stabilization are involved. They should investigate whether structural CIN from (e.g., acentric chromosome arms) or numerical CIN (intact chromosomes with one centromere and two telomeres) from chromosome mis-segregation is the prominent consequence in aged fibroblasts. For example, they should perform centromere-specific FISH and calculate the frequency of whole chromosome relative to chromosomal fragment containing micronuclei.

- The assessment methods of CIN should be improved to make it more unbiased and more quantitative. This is needed especially because the effect size is in many cases small (eg Fig.2c the increase of lagging chrs is ~1.7 folds between 3 % and 20% O2 in aged fibroblasts). CIN is currently assessed only by qualitative counting of abnormal structures, like lagging chromosomes and MN. The authors should either use an automated image analysis pipeline or use sequencing-based digital karyotyping.

- In Fig. 5g-i the authors provide evidence of the replication stress involvement in the CIN by supplementing cells with nucleoside and determining if there is decreased rates of MN and 53BP-bodies. They should exclude the possibility that decreased mitoses is responsible for the effect, by assessing the cell division rates or arrested cells in the rescue condition.

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- The authors should explain the surprising amount of damage observed in fibroblasts from young mice (around 5 foci/cell, Fig. S4a). Similarly, the young fibroblasts exhibit high baseline rates of 53BP1-body containing cells (~12 % of cells with 53BP-bodies, Fig. 5b). If it exists, the authors should cite literature showing that others have observed high baseline DNA damage in this cell type

Minor points:

- The authors should provide the distributions of the cells counted in the experiments, or the total number of cells counted per animal.

- The authors should comment on the two-fold discrepancy between the fixed and live imaging on the prevalence of the lagging chr and bridges in 3% O2 (Fig 1c and 2c). If this difference reflects variation between replicates, they should also explain the absence of heterogeneity between the individual animals used in some experiments (eg Fig 5d-i).

- line 166 and Fig.2: The authors should change the wording and should mention Â“ambient oxygenÂ” and not Â“increased oxidative stressÂ”.

- line 184: fix references

- line 38: Points mutation -> point

- line 224: typo Â“with ageÂ”

Comments for the author

Chen et al. suggest a link between oxidative stress and chromosome instability (CIN) in aging. They show that CIN emerges in the form of abnormal nuclear structures or defective mitoses in ex vivo cultures of primary fibroblasts from aged mice, but not from young mice. They furthermore propose that replication stress, a hallmark consequence of aging, is induced by oxidative stress and together with microtubule stabilization are the mechanisms that underlie CIN in aging.

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- line 184: fix references

- line 38: Points mutation -> point

- line 224: typo “with age”

First revision

Author response to reviewers' comments

Response to the Reviewer 1’s comments:

Overall the experimental part of this work is well executed and results are presented in a coherent and clear manner. The study is relevant for both the fields of aging and chromosomal instability. There are, however, some concerns over the novelty of the study. Chromosome missegregation, oxidative stress and aging in mouse fibroblasts have never been studied in the same model system

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(fibroblasts), but several of these links have studied separately in the past. It is known that oxidative stress causes chromosome missegregation in drosophila oocytes (Perkins et al 2016) and human-hamster hybrid cells (Limoli & Giedzinski 2003). Moreover, oxidative stress has been linked to aging in mice (Forster et al 1996, Navarro et al 2002) and rat (Gilmer et al 2010) brains. The authors should at minimum better discuss how their study advances the field, and cite those papers in their discussion.

We cited the papers indicated by the reviewer and discussed our study clearly demonstrated the link between aging, oxidative stress, and CIN in the same model system (line 361-366, 374-376).

Major points:
1. Throughout the authors refer to chromosomal instability in aging cells, but they never actually check for presence of aneuploidy in their model, and instead rely on indirect readouts such a lagging chrosomes and micronucleis. While those can lead to chromosome gain/loss it is not automatic. Since the authors report 6% of chromosome missegregation in aged cells (Figure 1C) one would expect to see an increase of aneuploidy in their model. Therefore the authors should compare the karyotypes of old and young fibroblasts.

According to the reviewer’s comment, we quantified the number of chromosomes in fibroblasts from young and aged cells in metaphase chromosome spreads. As shown in Fig. 1E in the revised manuscript, there was a significant increase of metaphases with abnormal numbers of chromosomes in cells from aged mice, confirming that chromosomal instability in fibroblasts from aged mice leads to the appearance of aneuploid cells (line 131-135). We also found that both metaphases with fewer and more than 40 chromosomes increased, while the average number of chromosomes did not change, which are shown in Fig. S1G, H in the revised manuscript (line 135-139).

2. All the rescue experiments presented in Figure 4 (oxidative stress), Figure 5 (replication stress) and Figure 6 (oxidative stress and stabilized microtubules) show partial and sometimes weak rescues. Altough the authors present their results in carefuly manner, their discussion nevertheless implies a causal chain with aging on top driving oxidative stress causing replication stress which in turns leads to stabilized microtubules. One caveat is that in their model the authors did not directly test whether replication stress is upstream of stabilized microtubules. Second, given that the observed effects are only partial, the authors should better discuss these partial rescues, or test whether alternative treatments yield clearer results. Would a combined NAC and Mito Q treatment give a better rescue? Would a direct inhibition of the DNA damage pathway, such as inhibition of ATR show a better rescue. Also, in terms of additive effect, would a combination of NAC and UMK57 give a non-additive effect, as implied by their model? Some of these experiments could put this study on a firmer footing, by providing additional controls and a certain redundancy.

In response to the reviewer’s comment, we observed whether a combined NAC and MitoQ treatment provides a better rescue. As shown in Fig. S4A in the revised manuscript, cells treated with NAC and MitoQ did not show further reductions in the rates of 53BP1-positive cells and micronucleation, compared to cells treated with NAC alone. On the other hand, the reduction was small but significant compared to cells treated with MitoQ alone. These data suggest that ROS produced not only in mitochondria, but also at other sites is responsible for the induction of replication stress and CIN in fibroblasts from aged mice (line 228-233, 307-311). We also observed whether a combination of NAC and UMK57 gives an additive effect. As shown in Fig.S4A in the revised manuscript, cells treated with NAC and UMK57 showed no further reductions in the rate of 53BP1-positive cells and micronuclei compared to cells treated with NAC alone, showing that increasing microtubule dynamics by UMK57 does not cause an additive effect with NAC treatment on replication stress and micronucleation. In contrast, the rate of cells containing micronuclei was further reduced in cells treated with NAC and UMK57 compared to cells treated with UMK57 alone, suggesting that microtubule stabilization partially contributes to micronucleation downstream of oxidative stress in cells from aged mice (line 330-338).

Minor points :
1-The images presented in Figure 2A have an insufficient quality, the manuscript would benefit from better examples.
Although the quality of the images is limited because they were taken at a low magnification (×20), we replaced the images of proper segregation and lagging chromosome in Figure 2A

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with those representing each phenotype more clearly.

2-Figure 3C would benefit from splitting the green and red channel in the presented images to better visualize the reduction in the red channel in old fibroblasts. It also would be useful to explain the meaning of red and green signal in the legend of the figure.

In response to the reviewer's comment, we splitted the channels in Figure 3C and explained in the legend what each color represents (line 900-903).

Response to the Reviewer 2's comments:
Major comments:

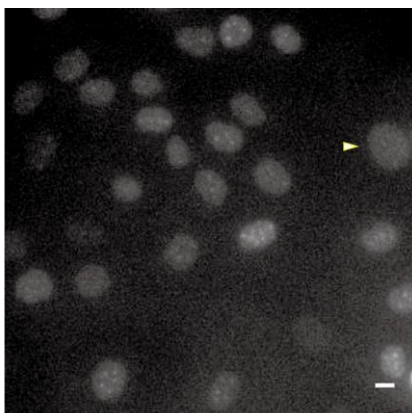
In terms of the overall significance, this paper does a nice job of connecting a series of "dots", but each pair of dots have already been connected in prior work. Although the overall conclusions are mainly confirmatory, in balance, we feel that the paper makes a valuable contribution. We particularly appreciate the experiments where cultures are grown in physiological normoxia. The authors show that oxidative stress and mitochondrial dysfunction induce replication stress and CIN in primary cultures of aged fibroblasts, as expected from prior work. However, since aneuploidy - which is a consequence of CIN - is a hallmark feature of aging (eg. Naylor and Deursen, 2016) and CIN has been suggested to be observed in models of aging (eg. see a review doi: 10.1002/mc.22539), the novelty of this work is limited to the linkage between oxidative stress, replication stress and CIN in aged fibroblasts in one experimental setting.

The methods, as well as the results, are clearly presented. The introduction should be improved by a better discussion of prior literature on CIN in aging and the novel points should be clearly stated. For example, the authors state that work on mosaic variegated aneuploidy syndrome is the only prior and indirect evidence of CIN in aging. Prior literature on aneuploidy in aging which is predictive for the underlying CIN should also be mentioned, as discussed above. In addition, the authors should clarify specific interpretations of their results (see specific points below).

According to the reviewer's comments, we discussed prior literature on CIN in aging in the introduction and the discussion, and emphasized the novel points of our study that it clearly demonstrated the link between aging, oxidative stress, and CIN in the same model system (line 76-78, 361-366, 374-376).

- the authors should assess the possibility that the effects in aged fibroblasts (eg increased MN) reflect the presence of tetraploid cells in the population (a common occurrence in cultures of primary mouse cells). Especially since Fig. S1b implies that this population is large (around 15-20%). All the comparisons between old and new fibroblasts should be restricted to near-diploid cells.

The difference in the percentage of tetraploid cells in young and aged mice at the second passage is not statistically significant (P = 0.1163, added in the revised manuscript). Cells with large nuclei, indicative of tetraploid cells, were easily distinguishable (see the image below). We did not include these cells in our analyses, which we mentioned in the revised manuscript (p. 5, lines 113-114).



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An image of mouse primary fibroblasts, in which nuclei were visualized by SiR-DNA. A cell with a large nucleus, indicative of a tetraploid cell, is indicated by an arrowhead. Scale bar: 10 μm.

- the type of CIN observed in aged fibroblasts should be further examined, which would help clarify the underlying mechanism. The authors propose that both replication stress and microtubule stabilization are involved. They should investigate whether structural CIN from (e.g., acentric chromosome arms) or numerical CIN (intact chromosomes with one centromere and two telomeres) from chromosome mis-segregation is the prominent consequence in aged fibroblasts. For example, they should perform centromere-specific FISH and calculate the frequency of whole chromosome relative to chromosomal fragment containing micronuclei.

To clarify whether micronuclei in fibroblasts from aged mice were derived from intact chromosomes or acentric chromosome fragments caused by chromosome breaks, we observed micronuclei for the presence of CENP-A, a centromere marker. As shown in Fig. 5D in the revised manuscript, both CENP- A-positive and negative micronuclei increased in cells from aged mice, with more than half of the micronuclei being CENP-A-negative, implying that micronuclei in fibroblasts from aged mice contain both whole chromosomes and acentric chromosome fragments, which are supposedly caused by replication stress (line 265-272). We also confirmed in metaphase chromosome spreads that both structural CIN and numerical CIN were present in aged fibroblasts (Fig. 1E and 5C in the revised manuscript, line 131-139, 263-265).

- The assessment methods of CIN should be improved to make it more unbiased and more quantitative. This is needed especially because the effect size is in many cases small (eg Fig.2c the increase of lagging chrs is ~1.7 folds between 3 % and 20% O2 in aged fibroblasts). CIN is currently assessed only by qualitative counting of abnormal structures, like lagging chromosomes and MN. The authors should either use an automated image analysis pipeline or use sequencing-based digital karyotyping.

In response to the reviewer's comment, we quantified the number of chromosomes in fibroblasts from young and aged cells in metaphase chromosome spreads. As shown in Fig. 1E and Fig. 5C in the revised manuscript, there was a 3-fold increase of metaphases with abnormal numbers of chromosomes and chromosome fragments in cells from aged mice compared to cells from young mice, confirming the presence of CIN in fibroblasts from aged mice (line 131-139, 263-265).

- In Fig. 5g-i the authors provide evidence of the replication stress involvement in the CIN by supplementing cells with nucleoside and determining if there is decreased rates of MN and 53BP-bodies. They should exclude the possibility that decreased mitoses is responsible for the effect, by assessing the cell division rates or arrested cells in the rescue condition.

We measured the mitotic indices in fibroblasts from young and aged cells in the presence or absence of nucleosides, and found that they were not altered in the presence of nucleosides, which is shown in Fig. S4G in the revised manuscript. The data exclude the possibility that decreased mitoses is responsible for the reduction of cells with 53BP1 foci, ultrafine bridge, and micronuclei in the presence of nucleosides (line 291-294).

- The authors should explain the surprising amount of damage observed in fibroblasts from young mice (around 5 foci/cell, Fig. S4a). Similarly, the young fibroblasts exhibit high baseline rates of 53BP1-body containing cells (~12 % of cells with 53BP-bodies, Fig. 5b). If it exists, the authors should cite literature showing that others have observed high baseline DNA damage in this cell type

The number of g-H2AX foci reported in the literature varies considerably, probably due to differences in the type of cells and the threshold of signal levels to be counted as foci. In several papers, relatively high numbers of g-H2AX foci were reported as endogenous DNA double-strand breaks. For example, in *Curr Biol*. 2000; 10(15): 886-895, around eight g-H2AX foci were detected in unirradiated IMR90 cells, a human fibroblast cell line (see Figure 3(a) below). In *Br J Cancer*. 2010; 102, 1511-1518, six g-H2AX foci were detected in unirradiated human lymphoblasts (see Figure 1A, F below). In *Int J Radiat Biol*. 2021; 97(5): 642-656, five g-H2AX foci were detected in unirradiated mouse skin cells (see Figure 2 below).

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Figure 3

Quantification of radiation-induced foci. (a) IMR90 cells, either unirradiated or irradiated with 12 Gy, were stained with γ-H2Ax and Rad50 antibodies, as in Figure 2a-c. At least 30 images were collected from each time point and the number of overlapping foci was determined using NIH Image. Foci were counted in this way for each time point: unirradiated (0) or irradiated (0.5; 2, 4, 6 and 8 h recovery). The sets of data from each time point exhibited Poisson distributions, and the average value for each data set is plotted on the graph. The Y axis represents the number of foci per average cell nucleus area. The Y axis represents the number of foci per average cell nucleus area. The number of γ-H2Ax-Rad50 overlapping foci (filled diamonds) is represented in comparison with the total number of γ-H2AX foci (open squares). (b) An analysis of γ-H2AX-Brca1 overlapping foci (open squares) is shown in comparison to Brca1-Rad51 overlapping foci (closed squares) at four different time points: 0 (unirradiated cells), 45 min, 2h, and 6h, using methods described in (a).

[NOTE: A figure provided for the reviewers has been removed. It showed Figure 3 from Paull et al. (2000), A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr. Biol. **10**, 886-895. doi: 10.1016/s0960-9822(00)00610-2]

Figure 1. Dose response of γH2AX focus formation in lymphoblast cell lines (LCLs). Immunocytochemistry (ICC) was used to capture representative γH2AX focus images from asynchronous log-phase non-radiosensitive (RS) control cells (control-5)before (A) and I h after I Gy (B), 2 Gy (C) and 4 Gy (D) of ionising radiation A more detailed view of yH2AX foci were quantified as foci per nucleus for each dose (F) Error bars are the standard error of the mean (s.e.m) of γH2AX foci numberper nucleus from three separate experiments, wherein at least 100 nuclei cells were scored per dose.

[NOTE: A figure provided for the reviewers has been removed. It showed Figure 1 from Vasireddy et al. (2010), H2AX phosphorylation screen of cells from radiosensitive cancer patients reveals a novel DNA double-strand break repair cellular phenotype. Br. J. Cancer **102**, 1511-1518. doi:10.1038/sj.bjc.6605666]

Figure 2. In mouse skin, the BB calibration curves of manual and macro foci counts were in close agreement with each other. Each data point reprsents the average number of foci per nuclei for all 6 mice in each experimental group. The error bars represent SEM for each group. The liner regression lines of foci counting (manual and macro methods) was generated and used to establish the yield of foci/cell/Gy. We established a yield of 5.6 + 0.42, 5.3 _ 0.25 and 5.9 + 0.04 foci/cell/Gy for manual and macro subset images, and macro foci counts for all images respectively. The correlation coefficient for each method was between R^2 =0.94 -1.

[NOTE: A figure provided for the reviewers has been removed. It showed Figure 2 from Ventura et al. (2021), The γH2AX DSB marker may not be a suitable biodosimeter to measure the biological MRT valley dose. Int. J. Radiat. Biol. **97**, 642-656. doi:10.1080/09553002.2021.1893854]

Regarding the rates of 53BP1-containing cells in Fig. 5B, they were measured on binucleated cells derived from cytokinesis failure by cytochalasin B. Therefore, these values should be halved to make them represent the rates of nuclei positive for 53BP1, which corresponds to the rates of 53BP1- containing cells in a physiological condition. The rate of 53BP1-containing nuclei from young mice in Fig. 5B is ~8% as shown in the graph below, which matches the rate in Fig. 5A.

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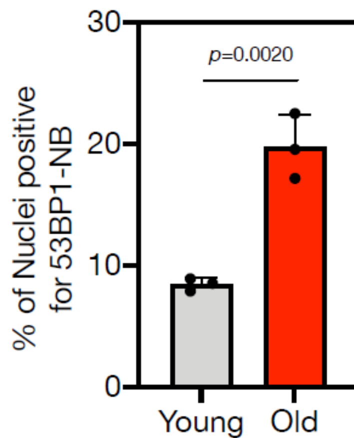

The rate of nuclei positive for 53BP1 nuclear bodies in binucleated cells shown in Fig. 5B. Error bars represent S.D. P-value was obtained using the Student's *t*-test.

Minor points:

- The authors should provide the distributions of the cells counted in the experiments, or the total number of cells counted per animal.

In response to the reviewer's comment, we provided the information of the distributions of the cells counted in the experiments in the figure legends.

- The authors should comment on the two-fold discrepancy between the fixed and live imaging on the prevalence of the lagging chr and bridges in 3% O2 (Fig 1c and 2c). If this difference reflects variation between replicates, they should also explain the absence of heterogeneity between the individual animals used in some experiments (eg Fig 5d-i).

In live cell imaging, we were able to detect lagging chromosomes and chromosome bridges even when they appeared transiently during mitotic progression. In contrast, in fixed cell samples, we were able to detect them only when they were present at the time of fixation, resulting in the reduced rates of lagging chromosomes and chromosome bridges in fixed cell samples compared to live cell imaging.

- line 166 and Fig.2: The authors should change the wording and should mention "ambient oxygen" and not "increased oxidative stress".

We changed the wording according to the comment (line 180).

- line 184: fix references

We fixed the references in a proper format (line 197).

- line 38: Points mutation -> point

We corrected the typo (line 40).

- line 224: typo "with age"

We deleted the duplicate phrase (line 244).

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Second decision letter

MS ID#: JOCES/2022/260688

MS TITLE: Oxidative stress induces chromosomal instability through replication stress in fibroblasts from aged mice

AUTHORS: Guan Chen, Zhenhua Li, Kenji Iemura, and Kozo Tanaka
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

This manuscript makes an interesting connection between aging, oxidative stress, replication stress and chromosomal instability

Comments for the author

The authors have addressed my concerns in full, and I therefore support publication of this interesting manuscript

Reviewer 2

Advance summary and potential significance to field

See my previous review.

Comments for the author

This paper has nicely addressed my prior critique and is now ready for publication.

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