Reviewer’s report

Title: Melanoma cells replicate through chemotherapy by reducing levels of key homologous recombination protein RAD51 and increasing expression of translesion synthesis DNA Polymerase ζ

Version: 0 Date: 15 Aug 2017

Reviewer: Bernd Kaina

Reviewer's report:

In this manuscript by Song et al. it is shown that treatment of melanoma cells with cisplatin results in downregulation of Rad51 and, concomitantly, upregulation of DNA polymerase zeta. The effect seems to be melanoma-line specific. The experiments are well performed and the manuscript is very well written. Before publication, the work needs revision as follows.

Major points

1. Decline in Rad51 has been shown on RNA level by RT-PCR and on protein level by Western blotting. Since this observation is very important, it's the key observation in this work, I suggest to substantate the finding by an experiment demonstrating RAD51 foci by immunocytochemistry. Cisplatin induces HR in S-phase cells, which should be accompanied by Rad51 assembly. If Rad51 is downregulated I expect a lower level of Rad51 foci in melanoma compared to non-melanoma cells (eg. human diploid fibroblasts) following cisplatin. I also recommend staining immediately after cisplatin treatment and 2 days later when Rad51 is down on protein level, which should make a difference.

2. The finding of cisplatin resistance of melanoma cells rests on an enhanced level of DNA polymerase zeta, which is upregulated by cisplatin. I'm wondering whether in non-melanoma cells transfection of pol zeta results in cisplatin resistance. Has this been shown by others? If not, can this control experiment be done, which would further support the model?

3. Knockouts for Rev3L, the catalytic subunit of pol zeta, are hypersensitive to fotemustine, a crosslink-inducing anticancer drug, used also for therapy of malignant melanomas. Authors concluded that pol zeta plays a role in the tolerance of crosslinks, thus enhancing survival (Roos et al., Mol. Pharmacol., 76, 927-934, 2009). This word should be discussed as it supports the data reported here.
4. In studies by others it was shown that pol eta is subject of upregulation by genotoxic stress. Is pol eta coregulated with pol zeta?

5. Repair of interstrand crosslinks (ICL) requires, to my understanding, both incision, TLS and HR, at least in S phase. In G1, ICL repair is less efficient and does not involve HR. Is the process of ICL repair in melanoma cells G1 or S or G2 phase dependent? Suppl. Fig. 1 nicely shows that Rad51 goes down on day 2 and 3 following treatment. The recombination or bypass process must become relevant at this very late stage. Are the cells in the treatment cell cycle for such a long time? A growth curve would be fine, showing whether the cells continue proliferation after the addition of cisplatin or whether a substantial proportion is arrested. If they are not arrested, TLS must be postulated to occur in the 2nd or even the 3rd cell cycle after lesion induction.

Minor comments

6. Page 5. Supplement Fig. 1 is most important. I strongly suggest to show the blots in the main part of the paper and the quantification in the supplement, or both in the main presentation; in this case the quantification can be given below each lane (below β-actin) in numbers (0 μM Rad51/β-actin = 1).

7. Suppl. Fig. 2 should also be shown in the main part. The original western is so important as it shows a) the quality of study, b) the loading control, c) the specificity of the AB. Quantification is often inaccurate because of saturation levels of band intensity, strong background and other factors.

8. Page 5, 2nd paragraph: "…, where cell showed an increase in Rad51 protein expression …" Is this the case in PE01 cells? Is control and 3 μM cispt significant different from each other? Please use statistics.

9. Page 5, fig. 3. Please arrange the cell lines in the same order as in fig. 2, starting with the melanomas.


11. Page 6, 3rd paragraph. "This tag has been used extensively …" Please, add a reference.
12. pol zeta expression was measured only in three melanoma lines (Fig. 6). What about HBL and WM 115 cells? Have they not been tested or do they not show an increase in pol zeta?

13. Page 9, 3rd paragraph. What is the plasma level in patients receiving intravenous infusion? Is the therapeutic concentration in the range of doses used in this work in vitro?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable
Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.