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: 16 Jul 2017

Reviewer: Brian Gabrielli

Reviewer's report:

This manuscript reports an interesting finding that the DNA damage repair protein RAD51 is commonly downregulated in melanomas as a response to Cisplatin treatment, but this downregulation was not observed in either ovarian cancer cell lines or fibroblasts. The downregulation appears to be through a reduction in transcript level rather than protein stability. The authors also examine the levels of two translesion synthesis polymerases, Pol zeta and Pol eta, and found that Pol zeta was selectively and strongly upregulated in parallel with RAD51 decreases, and this was only observed in melanomas. These interesting findings were then investigated in the context of sensitivity to Cisplatin, to assess whether the changes contributed to the lack of sensitivity of melanomas to Cisplatin therapy. The problem is that there is little data to support a contribution of either the RAD51 down-regulation or Pol zeta up-regulation to the Cisplatin insensitivity. Data for three non-melanoma cell lines are shown but none of these appear to be sensitive to the concentration of drug used, this correlation is not supported. Of course other mechanisms might be responsible for their insensitivity, but this does provide support for the hypothesis being tested in this study.

The cleaved Caspase 3 blot in Figure 5 shows that the RAD51 over-expressing cells already have quite elevated levels suggesting they already have a relatively elevated level of apoptosis, and this is also shown by the FACS data. So how well do the cells tolerate RAD51 over-expression? The FACS data also indicates a major change in the cell cycle profile with increased S/G2/M population and increased sub-diploid population, indicative of cell death. So is the increased cell death with Cisplatin treatment really because RAD51 levels are stabilised, or because over-expression of RAD51 is producing some sort of replication stress or DNA damage and this is exacerbating the effect of Cisplatin rather than the elevated RAD51 responding to the Cisplatin-induced damage. It may be necessary to establish an inducible expression system or express low levels of RAD51 to avoid the apparent intolerance of cells to the high level expression used here. The amount of cell death being driven is relatively small so it is difficult to accurately assess the relevance of the effects observed. Even the cell lines which do not down-regulate RAD51 appear relatively insensitive to Cisplatin. Do melanoma cell lines sensitive to Cisplatin fail to either down-regulate RAD51 or upregulate Pol zeta. What is the effect of cytotoxic doses of the drug on the non-melanoma cell lines? Is the difference simply a dose effect and higher doses of drug might produce the same changes in levels observed in the melanoma cell lines?
A number of important controls are missing. There is no untreated Flag-RAD51 over-expressing cell growth data. And in Figure 6, what is the effect of Pol zeta knockdown on viability without drug treatment?

>50 fold increase in control cell numbers in 3 days for the A375 cultures? That is almost population 6 doublings in 3 days, which is a very impressive growth. Is this correct? In my experience with a very large number of different melanoma cell lines, doubling times of 24h or greater are most common.

A375 are quite sensitive to olaparib so the increased sensitivity is relatively small 5 fold decreased IC50. As A375 is the extreme case in terms of effects on RAD51 reduction, is this increased sensitivity to Cisplatin with olaparib also the seen with the other melanoma cell lines? Bortezomib is a proteasome inhibitor similar to MG132, not a lysosomal inhibitor as suggested on p5.

**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.

No

**Are the conclusions drawn adequately supported by the data shown?**
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No

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