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: 18 Aug 2017

Reviewer: Michael Carty

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The incidence of melanoma, the most serious form of skin cancer, is increasing. However, treatment options are limited. One the main chemotherapeutic approaches, using platinum-based compounds such as cisplatin, is ineffective against melanoma. A better understanding of the mechanisms underlying cisplatin-resistance in melanoma cells could lead to new treatment options.

In the present manuscript, a series of melanoma cell lines were exposed to cisplatin and the expression of key DNA repair proteins was investigated. The main finding is that the level of the homologous repair protein RAD51 is decreased following exposure of melanoma cells lines to cisplatin. In contrast, Rad51 levels are unchanged or increased in control cells and in two ovarian cancer cell lines following cisplatin exposure. It was found that Rad51 levels can be stabilised using the protease inhibitor bortezomib, and that the decrease in Rad51 levels can be explained by decreased transcription of Rad51 mRNA.

In addition it was found that the levels the translesion synthesis protein DNA polymerase zeta (pol zeta) were increased in cisplatin-treated melanoma cells, and that siRNA-mediated knockdown of pol zeta reduced survival of cisplatin-treated melanoma cells. Synthetic lethality between cisplatin and the PARP inhibitor olaparib is reported in melanoma cells.

Main point

1. Rad51 is tightly associated with chromatin and forms nuclear foci at sites of DNA damage. To more clearly demonstrate that Rad51 protein levels are decreased in cisplatin-treated melanoma cell lines, western blots of whole cell lysates, and of fractionated cell extracts, showing nuclear and cytosolic fractions with appropriate protein markers, should be presented. This is essential to rule out the possibility that the observed reduction in Rad51 levels is due to failure to extract Rad51 protein that is tightly associated with chromatin in damaged cells.
2. The formation of Rad51 foci in cisplatin-treated cells should also be analysed using immunofluorescence, to determine whether the decreased levels of Rad51 protein on western blots are consistent with decreased formation of nuclear foci.

Minor point

1. Molecular weight markers should be included on all western blots (Fig. 5, Suppl. Figs 1, 2).

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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Yes

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