Reviewer's report

Title: Residual gammaH2AX foci as an indication of lethal DNA lesions

Version: 1 Date: 27 August 2009

Reviewer: Veronique A.J. Smits

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Banath et al BMC Cancer

This manuscript by Banath et al exits of two parts. In the first they describe the correlation between the fraction of cells that retained #H2AX foci 24h after treatment with different DNA damaging agents and clonogenic survival two weeks later.

In the second part they demonstrate that it is actually the majority of cells containing foci (Rad51-GFP in this case) that are the ones that do not survive the DNA damage treatment and therefore do not form colonies.

The authors have shown the correlation between IR-induced #H2AX foci and clonogenic survival in two earlier publications. Their new data therefore confirm these observations, using a wide range of DNA damaging agents (including for example the induction double strand breaks by processing of the DNA lesions or by turnover during DNA replication). The data showing that the fraction of cells that retained foci were the ones that lost clonogenicity is also not unexpected given their earlier publications, but is interesting, and an important novel direct observation. In addition, the set up of the experiment, tracking individual SiHa cells stably expressing Rad51-GFP for foci formation and colony survival is an elegant one.

However, I would like to see some comments addressed to improve the manuscript before I would recommend it for publication in BMC Cancer:

1. All ‘pilot experiments’ regarding Rad51 foci (Figure 5) were shown by staining for endogenous Rad51. Given the importance of the final experiment (demonstrating that the fraction of cells that retained Rad51 foci are the ones that loose clonogenicity), which is done with Rad51-GFP foci, these pilot experiments should be performed with Rad51-GFP as well, to demonstrate that the approach using Rad51-GFP is identical, or at least largely similar as for endogenous Rad51.

The reason for my concern is the fact that Rad51-GFP foci seem to disappear less quickly than foci of endogenous Rad51, as shown in Figure 6D. The authors state that at 24h after IR, the fraction of cells with Rad51 foci is the same for endogenous Rad51 and Rad51-GFP, but this conclusion is only based on one datapoint for endogenous Rad51, whereas there are many for Rad51-GFP. Vice versa, many time points were taken to measure endogenous Rad51 foci at early stages after IR (0-12h), whereas for Rad51-GFP there are only 2 ‘early’ time
points, 9 and 16h.
I therefore think a better comparison, taking early and late time points for both Rad51-GFP and endogenous Rad51, has to be made between these two approaches.

2. To strengthen their interesting observations summarized in Figure 6E, I would recommend the use of an additional dose of IR. Alternatively, a different DNA damaging agent, for example MNNG, could be used to confirm the data. The latter would also link both parts of the manuscript in a better way. Moreover, for clarity, I recommend representation of the data in a different way. For example by labeling the figure: ‘Rad51 positive: Surviving doublets/Total doublets’ and ‘Rad51 negative: Surviving doublets/Total doublets’ and below ‘% of survival’.

3. The aim and setup of experiments in the first part of the manuscript are only very briefly described. The manuscript would improve from explaining the idea behind and the conclusion of each of the experiments better. Also, the genotype and/or radiosensitivity of each of the cell lines used in Figure 5C should be explained in the text of the results section.
In addition, it is not always clear to this reviewer what assay is used. Examples are
- #H2AX formation in Figure 2B and C: foci formation analysed by IF or FACS?
- Remaining breaks in Figure 2F: what assay?
Finally, the authors name the data represented on the y-axis of Figures 2A, 3A/C, Figure 4 ‘Clonogenic survival’, ‘Surviving fraction’ and ‘Clonogenic surviving fraction’ respectively. As I assume it is all the same assay, the clearest way would be to label the axes in all figures the same way.

4. The data of Figure 1 could be represented better to show the described effect, that #H2AX formation after low doses of MNNG is limited to S phase cells. The figure would improve by expanding the x-axis and plotting less cells in each dot-plot, so S phase cells can be better discriminated.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests