Reviewer’s report

Title: The radiosensitising effect of gemcitabine and its main metabolite dFdU under low oxygen conditions is in vitro not dependent on functional HIF-1 protein

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Reviewer: Sonia Rocha

Reviewer’s report:

In this study, the authors investigate the role of HIF-1 in the radiosensitisation of MDA-MB-231 cells to gemcitabine and dFdU in hypoxia. They use a dominant negative overexpression system and state no differences observed.

Major Compulsory Revisions

Although, it is very likely that the system used works, the authors do no present enough evidence that the dominant negative is the same as not having no HIF-1. It is clear from recent findings that HIF-1 has non-canonical functions, that do not require HIF-1b, and possible no transcriptional activity. As such the system used cannot be stated to be the same as loss of function HIF-1. The authors should provide more evidence for the system used. The blot provided for CA9 is of poor quality and does not seem to show much difference to the levels of this target if you have wildtype or mutant HIF-1. Similarly, VEGF secretion is only minimal and it not obvious if this is relevant at all biologically. Also the authors say they performed a qPCR array but do not show any changes in genes apart from 1. This really needs to be demonstrated.

The authors should perform some of their experiments with siRNA depletion of HIF-1 instead and compare it with the system used. I would not ask that all experiments be done this way, but the cell cycle analysis or survival analysis would be required.

The data on survival is presented in a difficult to understand figure. The authors could split into several panels and provide a side by side comparison of wildtype and dnHIF results.

Also the cell cycle analysis comparison is not clear. Did the authors compare wiltype/EV to dnHIF stage by stage? There seem to be some substantial differences in the numbers provided with the different treatments, especially using the higher doses.

Minor Essential Revisions

The data is presented in a confusing manner and should be simplified. Also the statistical analysis should be done wiltype/Ev to dnHIF side by side for each condition used. It is not clear if this is case.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare no competing interests