Author's response to reviews

Title: Siah1 Proteins Enhance Radiosensitivity of Human Breast Cancer Cells

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Author's response to reviews: see over
Dear Editor,

Please find enclosed our revised manuscript entitled:

“Siah1 Proteins Mediate Radiosensitivity of Human Breast Cancer Cells”

by He et al., which we request for revision, upon second submission, in BMC Cancer.

We would like to thank the editor and the reviewers for the useful comments and suggestions. We understand the comment and the concerns of the second reviewer. We have made further corrections, marked with red, which are shown below.

Please note that below I report the exact place (page and paragraph) of the modifications performed on the text, as they appear in the version of the manuscript that still contains all the corrections marked with red.
Reviewer #2:

1st comment: The authors did have a lot of revisions to improve the quality of the ms. However, the RNAi experiments was not done in the standard way. At least a pair of different and effective siRNA are required to exclude the possibility of 'off target' effects. In some cases, it also needs the 'rescue' experiment. I would give the authors one more chance and let them do one more experiment, using another siRNA to knock down Siah1 expression and see if both siSiah1s would suppress the radiosensitivity of cancer cells used.

Answer to the 3rd comment: We would like to thank the reviewer for the constructive comment. In the previously submitted version, we reported on the results of Siah1 silencing by using the main Siah1 siRNA (5'-AACTCCTGCCTCCTTATGTATTT-3').

To address the concern raised, we initially analyzed the expression of Siah1 protein after silencing its function with the Siah1 siRNA-2 and siRNA-3 (Page 10, last paragraph). Immunoblotting showed blockade of Siah1 protein in MCF-7 cells. Please note that we added the new images next to the previous western blot image (Figure 1C).

In order to exclude the possibility of “off-target” effects, the 2 new siRNAs (Siah1 siRNA-2 and siRNA-3) were used and the clonogenic survival of the MCF-7 cells was investigated, as suggested by the reviewer (Page 5, 2nd paragraph). Similarly to the main Siah1 siRNA, siRNA-2 and siRNA-3 resulted in significantly reduced radiosensitivity in MCF-7 cells and only minor variations, at the different radiation doses, were observed between the different siRNAs (Page 11, 2nd paragraph). The new clonogenic curves were added to the previous figure (Fig. 3B). The figure legends were also modified accordingly (Page 24, 1st paragraph and page 25, 1st paragraph).

Thank you for considering our revised manuscript for publication. I am looking forward to hearing from you.

Yours Sincerely

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