Reviewer's report

Title: Enhancement of the Radiosensitizing Effect of Temozolomide: Targeting EGFR-associated Signaling in Malignant Glioma Cells

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Reviewer: Peter Sminia

Reviewer's report:

General comments

The manuscript by Choi et al. concerns an important issue in the therapy of high-grade glioma patients: their resistance to radiotherapy (and chemotherapy), and how to improve glioma therapy by targeting therapy. The laboratory observations on cell lines are interesting. However, more details should be given in the Materials and Methods chapter. Despite the large number of experimental data that is presented, this reviewers' main question is the reproducibility of the results, as well as the analysis and quantification of the experimental data. I have serious concern regarding the (controls of the) clonogenic cell survival data and presented enhancement ratios. The discussion part of the manuscript, should be revised thoroughly.

Specific comments

Title: The title of the manuscript is not representative for the presented work. "Enhancement of the Radiosensitizing Effect of Temozolomide" is not or only partially covered by the presented data. Also, HDAC inhibition is not only EGFR-associated. Please change the title.

Abstract:
The results section should be revised (see comments below).

Background:
The paper of Stupp et al. (2005) is not a recent paper. In the meanwhile, long follow-up data are published (Stupp et al., Lancet Oncology 10: 459-466, 2009), which should be mentioned. With regard to the genetics of glioma, the next reference could be incorporated into the text: Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 23;455(7216):1061-1078, 2008.

The rationale for using HDAC inhibition as well as ligand-independent modulation as enhancement strategy should be explained in more detail, or by referring to the literature.

The final para (page 4) of the background chapter contains a conclusion as mentioned in the abstract, and should be deleted here.

Materials and Methods
Para Cell culture: details about treatment of cells with TMZ should be moved to the para Clonogenic assays.

Para Clonogenic Assays:

The concentration of 25 microM TMZ has no effect at all on the MGMT unmethylated T98 glioma cell line (literature data, and data from this reviewers lab), while it is a cytotoxic dose on U251 and U87 cells. Why was the TMZ dose not adapted to the sensitivity of the three cell lines to the drug, by taking an isoeffective drug dose? See also my later remarks.

Please add irradiation details. X-rays or gamma rays? Dose rate?

Para Wound healing assay

How was exactly the cell migration distance scored? How many wells were counted. Please add details.

Para Statistical Analysis

Were the experiments performed in triplicate, i.e. three independent experiments and were controls included in each separate experiment? Were three independent experiments performed for all the assays?

Results / Figures

Please move text and belonging references (e.g. second para, lines 1-5 and several other places) to the appropriate chapter of the manuscript, either the introduction or discussion.

This referee has serious concern regarding the clonogenic cell survival data presented in figure 2, and sensitizer enhancement ratios given in Supplementary table 1. As can be seen in that table, the SERs of TMZ alone groups, for all three cell lines, are the same. Does this mean that the cell survival assay with TMZ was performed only one time, and not repeated for each separate experiment with addition of the test drug, either RPM, PI103, 17DMAG or LBH? Each individual experiment should have its own TMZ + irradiation control curve. Since the IR alone and IR + TMZ cell survival curves presented in Figures 2ABCD are exactly the same for U251, as well as for the T98 cell line, this was probably not done. Because the additional effect of the three drugs were compared with the TMZ + IR curve alone – and enhancement ratios as presented in the supplementary table 1 calculated on basis of those curves, the obtained SER values and conclusions, if not performed with controls in each separate experiment, might be wrong.

Please comment on this main objection.

Figure 3A: the photographs are not convincing. Please add quantitative data.

Figure 4B: the quantitative data presented in this figure do not show a radioenhancing effect of TMZ alone. The compound PI103 seems to protect for the effect of IR, if correct: is the effect significant?
Figure 4C: The presented fluorescence photographs require better explanation in the text. It is difficult to see that addition of TMZ to each of the combination treatments of drug with irradiation enhances the lysosomal localization of LysoTracker.

Figures 5A + B: No radiosensitizing effect was found of TMZ additional to IR. How exactly was cell migration scored? Because the test took 24 hours, the effect of (drug induced inhibition of) cell proliferation might result in a higher reduction of cell migration in the test groups where drugs were present. How about the effects of the drugs alone, without TMZ and or IR? Please comment.

Figure 5C: Addition of TMZ has probably no extra effect on all combinations, and also no radiosensitizing effect. Quantitative data are required to draw a definite conclusion.

Results, Page 14 (see also my earlier remark:) move literature data to the introduction or discussion.

Discussion
This discussion in its present form is rather an overview of the literature than a discussion of the own findings, and placing those into perspective of current knowledge in this field of research.

The discussion should start with a short summary of own findings, the main message from the – in the results section - presented experimental data. Then, own data and data from the literature should be criticized and discussed. The main message from own data, future steps and clinical relevance should be part of the discussion. Taken together, the discussion has to be rewritten.

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

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

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