Reviewer’s report

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

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Reviewer: Chann Lagadec

Reviewer’s report:

I reviewed the manuscript from Choi et al. untitled “Enhancement of the radiosensitizing effect of TMZ: Targeting EGFR-associated signaling in Malignant Glioma cells”. The authors used inhibitors (rapamycin, PI103, 17DMAG and LBH589) which could affect a well described pathway involved in GBM radioresistance, EGFR, on 2 GBM cell lines to identify a possible radiosensitizer. After analyzing the sensitizer enhancement ratio (SER) authors used several assay to determine the effects of combined therapies on DNA damages, apoptosis, autophagy, invasion, migration and vasculature mimicry (VM).

Interestingly, rapamycin does not affect radiosensitivity of cells while inhibition of PI3K/mTOR (PI103), of HSP90 (17DMAG) or of HDAC (LBH589) seems to potentiate the combined treatment of radiation and TMZ, decreasing colony formation, migration/invasion and VM, and increasing apoptosis and autophagy.

Major comments:

While enhancement of radiosensitizing effect is clear for U251, it’s less clear for T98G. It would be interesting to have a way to statistically prove it...

Authors analyzed biological effects on the more affected cell line, U251. Since the effects are reduced on T98G, it would be interesting to check why? This could bring new targets to bypass more efficiently the resistance. Please show effect on gamma-H2AX, Apoptosis, autophagy, invasion, migration and VM for T98G

Most of the biological effects have been presented as pictures, without any quantification... please quantify them (counting or better FACS).

Authors never explained why they picked up 6Gy to analyze biological effects of combined therapies... is it the dose for best SER?

In all experiments, the control TMZ+drug(s) only are missing.

Authors claimed that RPM increases expression of LC3-II, as well as 17DMAG and LBH589... unfortunately, it’s not obvious at all on presented western blot... please provide a quantification bar graph of the 3 repeats.

To test potential side effects of combined therapies, authors used normal astrocytes irradiated with 2 Gy... unfortunately, even with malignant GBM, no effect has been observed at this dose... please do a dose effect or use 6 Gy as used for malignant GBM.
Authors never specified how they chose the dose concentrations of each drugs? Since RPM does not have effect, is the dose sufficient? RPM seems to induce autophagy but does not have effect on radiosensitivity... authors should use this opportunity to discuss about potential mechanisms differences or main pathways involved which should be explored to bypass GBM radioresistance...

Minor comments:
Authors used siRNA... this has never been described in material and methods. How long after siRNA transfection did you perform the western blot and the clonogenic assay? Since you don't observe a decrease of p-Akt, that might be due to phosphorylation induced by transfection-stress... please use an additional control: untransfected cells. Authors claimed that rapamycin-treated cells exhibit a staining for EphA2 as strong as the control or TMZ-treated cells... nevertheless according to the picture (and the western blot), RPM reduces expression of EphA2... Please quantify the staining.

“low level of MGMT expression, which indicated a high level of methylated MGMT”... has to be change by: “low level of MGMT, as previously described (ref) which might highlight a high level of MGMT promotor methylation”.

**Level of interest:** An article of importance in its field

**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