Author's response to reviews

**Title:** MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol

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**Author's response to reviews:** see over
Dear BioMed Central Editorial Team, MSc Natalie Pafitis:

I’ve supplement all the information according to your suggestion and our reply to the review’s comments is listed below. It has cost you so much time and energy to take care of the manuscript for the past few months. I would like to thank you for what you have done on behalf of the other authors of the paper. We really appreciate your help!

May I take this opportunity to thank you for guide?

Sincerely yours,
Chun-sheng Kang

**REFEREE#1'S COMMENTS 1:** In figure 6B(right), the scale is wrong and must be corrected. The unit shifts in the figure though there are results of 48.7%#70.1% shown in the text.

**Answer:** According to the referee’s suggestion, we make a minor alternation of Fig.6B and the revised graph is as follows.
REFEREE#1'S COMMENTS 2: In figure 5B, the number is not described and must be added.

Answer: In fact, Fig. 5B shows the percentages of apoptotic cells in the histogram. In order to make it easier to understand, the revised section is as follows which is substituted in the new version manuscript.

FACS analysis was performed to detect DNA fragmentation in apoptotic cells following combined use of miR-21 inhibitor and taxol in U251 and LN229 human brain cancer cells (Fig. 5A). Untreated cells served as a negative control. Percentages of apoptotic cells are shown in the histogram (Fig. 5B). Compared with single taxol (6.24% and 6.25%, respectively) and miR-21 inhibitor (21.75% and 18.74%, respectively) treatment in U251 and LN229 cells, the combination of the miR-21 inhibitor and taxol therapy caused a significant (p < 0.05) increase amount (24.68% and 21.97%, respectively) of apoptotic death, suggesting that an additive induction of apoptosis developed in the cells co-infected with the miR-21 inhibitor and taxol.
REFEREE#2'S COMMENTS 1: Interaction of miR-21 and taxol was mentioned multiple times, yet there is no data to support it? Interaction? normally means physical interaction?.

Answer: In the current manuscript, “interaction” means the physical interaction between miR-21 inhibitor and taxol, in other words, the addition of drug combination. For the sake of making it much easier to understand, “interaction” is replaced and “combination effect” is used instead.

REFEREE#2'S COMMENTS 2: There is no discussion on why miR-21 has similar apoptosis effect on U251 (Pten-mutant) cells compared to LN229 cells (Pten-wild type) given that Pten is the target gene of miR-21 in this study

Answer: Our previous research indicated that antisense miR-21 ODN could induce U251 and LN229 GBM cell apoptosis via attenuating EGFR signaling pathway. Besides, multiple cancer cell apoptosis or metastasis related genes including PDCD4, P53 signaling network, RECK, S-TRAIL etc were validated to be miR-21’s function targets in both brain tumors and other epithelium original human cancers. Presumably, miR-21 inhibitor mediated human GBM cell apoptosis effect in a one hit multiple target mechanism rather than directly inhibition of PTEN mRNA translation. Mild apoptosis induction difference of miR-21 inhibition in U251 and LN229 GBM cell suggested, compared to miR-21 blockage, PTEN wide-type or induction was a fine tune in the oncogenesis of GBM.

Following the instruction, the “Discussion” section, part “miR-21 inhibitor enhances anti-proliferation effect of taxol to glioblastoma cells independent of PTEN status” has been modified and rewritten as below.

Yet, it is worth noting that cytotoxicity data algorithm results indicated that the
miR-21 inhibitor additively interacted with taxol on U251 cells and synergistically on LN229 cells for MTT assay and additively for Annexin V/PI apoptosis assay in both GBM cell lines. Interestingly, the data of miR-21 inhibitor suppressed U251 GBM growth indicated there was an independent PTEN pathway although the exact mechanism was not clear. The above data suggested that both in the PTEN mutant and in the wild-type GBM cells, miR-21 blockage could increase the chemo-sensitivity to taxol. Chan et al reported that knocking down miR-21 could increase caspase3/7 activity similarly though in LN229 and U87 GBM cell that had different PTEN background [14]. Our previous research indicated that antisense miR-21 ODN could induce U251 and LN229 GBM cell apoptosis via attenuating EGFR signaling pathway. Besides, multiple cancer cell apoptosis or metastasis related genes including PDCD4[10], P53 signaling network[11], RECK[20], S-TRAIL[27] etc were validated to be miR-21’s function targets in both brain tumors and other epithelium original human cancers. Presumably, miR-21 inhibitor mediated human GBM cell apoptosis effect in a one hit multiple target mechanism rather than directly inhibition of PTEN mRNA translation. Mild apoptosis induction difference of miR-21 inhibition in U251 and LN229 GBM cell suggested, compared to miR-21 blockage, PTEN wide-type or induction was a fine tune in the oncogenesis of GBM. And miR-21 suppression had clinical potential to enhance chemo-drug effect of chemotherapy in GBM patient with different PTEN genetic background.