Reviewer’s report

Title: Valproic acid-induced amphiregulin secretion confers resistance to temozolomide treatment in human glioma cells

Version: 0 Date: 02 Feb 2019

Reviewer: Chang-Han Chen

Reviewer's report:

In this manuscript, the author found that AR expression regulated by VPA was involved in glioma tumorigenesis. Targeting AR activated by VPA could make glioma cells more sensitive to TMZ treatment. This study is interesting. However, to improve the quality of this manuscript, some issues need to be addressed.

1. In figure 1, VPA enhanced the cytotoxic effect of TMZ in U87MG cells. The data indicated that U87MG cells treated with TMZ/VPA increased the sub-G1 population only 1.2% compared to TMZ-treated cells. This data suggested that expect apoptosis pathway, VPA enhanced TMZ-elicited cell death might go through other pathways. Whether autophagy pathway (markers) was involved in TMZ/VPA-induced cell death? Furthermore, use one cell line was not enough to demonstrate the conclusion. The author should confirm this result in another cell line. In figure 1C and D, the protein levels of pro-caspase 3 and pro-PARP should be presented in the same panels in the figure.

2. In figure 2, AR, EGFR, TGF-β2, and VEGF were increased in VPA-treated cells, compared with the control. The intensities of these 4 proteins in VPA-treated cells were similar. However, the author did not demonstrate why AR was selected for further study. Please confirm if these 4 protein expressions in VPA-treated cells were higher than that in control group.

3. As shown in figure 2B, VPA decreased the AR protein expression level in VPA-treated cells. Whether VPA influences the AR mRNA expression level? In addition, if cells treated with VPA influence the AR protein stability?

4. In figure 3B, the shcontrol group treated with TMZ in 500 µM at 48 hour that the cell viability had 75%. In addition, using the same panel, the cleavage caspase-3 and PARP were also diminished. These results were not consistent as shown in figure 1. It is suggested that cell transfected with shcontrol combining with TMZ treatment might increase the cell viability and decrease the cell death. Please confirm the results and explain it.

5. In figure 3D, how long did cells treat with rAR? Only 8 hour? Please demonstrate it clear in the figure legends.

6. How did author get the U87MG-S cells? Please descript it detail in material and method. In addition, whether cell viability was influence in U87MG-S cells treated with VPA?

7. In figure 4E, why cells treated with TMZ with 1000 µM? This dose was not consistent with previous data.

8. Whether VPA combined with TMZ treatment will decrease the tumor growth in vivo?
Minor revision

1. What is "AR" mean? Please addition this information when it was appear in the first time.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

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