Reviewer’s report

Title: The JNK signaling pathway plays a key role in methuosis (non-apoptotic cell death) induced by MOMIPP in glioblastoma

Version: 0 Date: 23 Oct 2018
Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?
Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?
No - there are minor issues

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?
No - there are minor issues

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?
No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Could an appropriately REVISED version of this work represent a technically sound contribution?
Maybe - with major revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: This is an interesting study exploring the role of JNK signaling pathway in induction of methuosis by indolyl chalcones in glioblastoma cell line U251. The in vivo experiments add value to the observations and interpretation. Here are some of my concerns/suggestions to further improve the presentation -

In Figure 2B, authors have shown glucose uptake in a number of cancer cell lines which definitely provides confidence that the phenomenon is not cell-line specific. However, is it possible to show a few more preliminary experiments in cell lines other than U251 so that the cell line-specific effects are comprehensively ruled out.
In continuation of the above concern, have the authors tested the effects of compounds in a 'normal' non-tumorigenic cell line, so as to establish cancer cells-specific action of compounds.

While the concept of 'methuosis' is novel and interesting, I do not notice any apparent experiments to rule out apoptosis as the mechanism. This is further complicated by the knowledge that JNK signaling very much takes part in apoptosis as well. This, in this reviewers opinion, is critical and central to the hypothesis and needs to be clarified. Further, several other factors evaluated in the study, such as, Bcl-2 and Bcl-xL, are also very much part of apoptosis. As such, how can apoptosis be ruled out.

In light of above concern, authors need to identify and show molecular markers that are extremely specific for 'methuosis' with ideally no overlap with apoptosis or even autophagy.

The mention of 'indolyl chalcones' in the title is a little misleading because the only compound being investigated is MOMIPP, with MOPIPP serving just as a non-toxic control. If the article would have started with a screening of compounds followed by mechanistic studies with the most potent compound, this title would have made more sense but clearly authors have published screening part earlier and this study was focused on MOMIPP.

After writing the last comment, I notice two more chalcones used in the study - 2q and 2a. 2q seems to functionally similar to MOMIPP while 2a seems to be functionally similar to control MOPIPP. My concern now is that why these additional compounds introduced in Figure 4? How about including them in Figures 1 through 4?

The pharmacokinetic data is promising. However, isn't 80 mg/kg dose quite excessive? What would be an equivalent dose in human?

Also, the effects of MOMIPP on tumor growth are shown. However, is it possible to corroborate in vitro findings i.e. show similar effects on signaling pathways in tumor remnants?

REQUESTED REVISIONS:
Please see my detailed comments above.

ADDITIONAL REQUESTS/SUGGESTIONS:
I trust that there are several points that need clarification, and some additional controls and experiments will certainly improve the quality as well as the confidence in findings.

Note: This reviewer report can be downloaded - see attached pdf file.

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

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
No

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

No

**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.

Not relevant to this manuscript

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

Acceptable

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