Reviewer’s report

Title: Valproic Acid Sensitizes Metformin-resistant Human Renal Cell Carcinoma Cells by Upregulating H3 Acetylation and EMT Reversal

Version: 0 Date: 25 Oct 2017

Reviewer: Anindita Chakrabarty

Reviewer's report:

The goal of the reported study was to test 1. Chronic metformin treatment induces resistance in RCC cells and 2. metformin resistance in RCC can be reversed by combining it with a histone deacetylase inhibitor valproic acid (VPA).

While searching for the molecular mechanism that might contribute to metformin resistance, the authors identified up regulation of EMT markers, down regulation of pAKT, pAMPK and acetylated H3 (aH3). Combining VPA with metformin reversed these phenomenon. The authors implicated that metabolic reprogramming by pAMPK and pAKT determines the changes in histone acetylation levels in resistant cells which in turn affects EMT. Several points need to be addressed for consideration for publication in BMC Cancer. These are:

1. Authors should improve the english writing for clear representation of facts and findings.

2. Authors should check cross resistance of metformin R line with similar class of drug (e.g. phenformin or related AMPK activators). This will help to deduce if the resistance is due primarily to modulation of AMPK activity following chronic metformin exposure.

3. EMT is often associated with gain of stem-like marker expression and functional properties of stem-like cells. Authors should check whether EMT induction in metformin R line is correlated with acquisition of stem-like phenotypes and whether this can be reversed by HDAC inhibitor like VPA.

4. Authors should check whether another general HDAC inhibitor like TSA or vorinostat can be as effective as VPA against metformin R cells. This will confirm whether reversal of metformin resistance is truly associated with change in aH3 levels.

5. Authors should include a pictorial representation of tentative roles of aH3, pAMPK and pAKT in conferring metformin resistance in RCC cells.
   Common: major revision required.
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:

Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal