Author’s response to reviews

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

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Author’s response to reviews:

Dear Dr. Chakrabarty:

Thank you very much for giving us the opportunity to revise our manuscript entitled "Valproic acid sensitizes Metformin-resistant human renal cell carcinoma cells by upregulating H3 acetylation and EMT reversal", manuscript ID BCAN-D-17-01686R1. We also want to thank for the reviewers for their detailed comments. We have studied their comments carefully and have made correction which we hope could meet with their approval.
Metformin is a diabetic drug which obtained large amounts of attentions in studies about cancer. In many laboratory studies, researchers found that metformin has remarkable antitumor activities in kinds of cancer. It is reasonable that researchers began to test the possibility of using metformin as an antitumor drug. But, we found that epidemiologically studies showed that the risk of cancer may not significantly associated with metformin therapy. This finding may hinder the using of metformin in cancer therapy. Why the results about the metformin antitumor effects in laboratory studies and epidemiologically studies are different? In the present study, we innovatively presented an explanation of this inconformity that long-term use of metformin induces resistance in cancer cells and we suggested that VPA can sensitize the metformin-resistant cancer cell. We believe our conclusions could provide meaningful arguments and novel perspectives to the studies about the relations between metformin and cancer.

We noticed that Prof. Ranganathan (Reviewer 3) suggested us that if metformin was not an alone treatment for RCC, then the rationale of using metformin in our present study may not clear. We completely agree with this valuable suggestion by the reviewer. It is a truth that for now metformin cannot be used as a standard alone treatment for RCC. But lots of researchers observed remarkable antitumor effects of metformin in their studies and tried to promote the use of metformin in cancer therapy. Like we discussed before, we believe our study provide meaningful and novel perspectives of the relations of metformin and cancer and will rise interests to the readers of your journal.

We want to thank for the times and attentions you gave for out manuscript. Hope we can publish our results in BMC caner.

Best wishes,

Qinghua Xia

P.S. We tried our best to do language corrections according to the reviewers’ comments in this revision. It is a shame that we cannot make sure there is not mistake in language because English is not our mother tongue. We send our manuscript to AJE for a standard English editing before, but after that we rewrote some parts of the manuscript in the R1 revision. The standard English editing only provided us one chance to upload out manuscript, so we chose to check the language three times by three different persons. If there are some minor mistakes in our revision, we hope
we can acquire the free assistance from your English language tutorial. But if an AJE editing is still needed, please let us know. Thank you very much.

Response to Reviewes:

Ekta Khattar (Reviewer 1): Most of my comments are answered except language which still needs more corrections.

#R: We have made language corrections according to the reviewers’ comments.

Prathibha Ranganathan (Reviewer 3): 1. As I had mentioned previously, the rationale for using metformin is not very clear. I agree that the authors have made an attempt to clarify, but the point is still not addressed. Is Metformin given as a treatment for RCC? If not, then what is the physiological relevance of this study? When you say sensitivity to metformin, what happens to the glucose levels in these patients?

#R1: We completely agree with this valuable suggestion by Prof. Ranganathan. It is our mistake that we may misunderstood the comment 1 about the rationale. We are sorry that we may not clearly expressed the antitumor effects of metformin in our manuscript.

It is a truth that for now metformin was not be used as an alone treatment for RCC. But like we mentioned in introduction part (Line 61-74), a lot of laboratory studies observed remarkable antitumor effects of metformin in kinds of cancer (Reference 6-13), including RCC (Reference 27-32). Metformin may be a potential antitumor therapy of RCC.

Unlike in laboratory studies, epidemiologically studies in patients with type 2 diabetes showed that the risk of cancer may not significantly associated with metformin therapy. This finding may hinder the using of metformin in cancer therapy. Our study tried to explain why the results about the metformin antitumor effects in laboratory studies and epidemiologically studies are not accordant. In the present study, we innovatively presented an explanation of this inconformity that long-term use of metformin induces resistance in cancer cells and we suggested that VPA can sensitize the metformin-resistant cancer cell. We believe our conclusions could provide
meaningful arguments and novel perspectives to the studies about the relations between metformin and cancer.

As for the glucose levels in patients, we thought these may not apply to our present study because we used cell lines as our research objectives and we did not study cancer metabolism. But in these studies about the cancer risks of patients use metformin, the glucose levels were controlled in an acceptable range according to diabetes treatment guidelines.

2. The bar graphs for determining IC50 in Fig 1, is better than previous one. Tabulation may be a better idea.

#R2: Thanks for the reviewer’s suggestion. We agree with the idea that tabulation may be better. But considering that bar graphs may more clear and direct to show the trend and we provided the exact values of IC50 in the manuscript, we hope Prof. Ranganathan could accept we still use the bar graphs.

3. The changes in the cell cycle profiles are not very convincing. It is better to get a statistician's opinion on the significance of these changes.

#R3: To determine whether the cell is arrest in G0/G1 phase, we settled two time points (24,48h). The data we analyzed are the percentage of G0/G1 cell after treatment. Like we described in methods and results sections, the experiments were performed more than three times and the results were analyzed by ANOVA and Paired T test.

4. In figure 3, the differences between VPA alone and combination are not very clear. Again, statisticians opinion may be useful

#R4: Thanks for the reviewer’s suggestion. In figure 3, the experiments were performed in 786-M-R cells. 786-M-R were resistant to metformin but not VPA. Our results showed the antitumor
effects of VPA were still existed in 786-M-R, so the differences between VPA alone and combination are not very clear.

5. In figure 6, the effect of TSA is not as apparent as VPA. Please comment on that.

#R5: Based on previous studies and our present study, we suggest that the effects of VPA were show in two pathways: the upregulation of acH3 and the upregulation of SMAD4. TSA is another kind of HADCi. As shown in fig. 6d, TSA and metformin combination could remarkably inhibit the level of pAKT, but show no significant effects on EMT markers. We speculate that because VPA can reduce the expression of SMAD4, therefore down regulate the level of pSMAD3/SMAD4, while TSA show no influence on SMAD4. (Line 303-308)

6. Fig 7 legend is missing

#R6: Thanks for the reviewer’s suggestion. We added the legend of Fig 7.

7. Fig 8 is not very clear. Legend is also missing.

#R7: Thanks for the reviewer’s suggestion. We added the legend of Fig 8.

8. the language needs to be better before publication.

#R8: We have made language corrections according to the reviewers’ comments.