Reviewer's report

Title: Carnosine inhibits the carbonic anhydrase IX-mediated extracellular acidosis and reduces the growth of HeLa tumor xenografts

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Reviewer: Xiaoguang Fang

Reviewer's report:

The manuscript titled “Carnosine inhibits the carbonic anhydrase IX-mediated extracellular acidosis and reduces the growth of HeLa tumor xenografts” is interesting. The authors studied the effect of carnosine on the tumor growth and presented their own point in this manuscript. But there are more experiment needed to be performed.

Major concern,

1. Since the work is to check effect of the carnosine on the tumor cell, it is important to know whether carnosine cause damage to the normal cell. Author should do some similar experiment on some normal cell.

2. To better understand the mechanism that carnosine retart tumor growth, it is important to check whether CA IX is a key element or not. The following experiment could be made. First knockdown CA IX with siRNA or shRNA, then doing the carnosine treatment experiment to check if it still reduce the tumor growth.

1. The measure methos is obscure. And there is no error bar in each time point. TTest should be made among each concentration, then we can know if there is significant difference between control group and the treatment group in Figure 1A.

2. There is no control in the treatment on SiHa, Hela, HT-29. #pH is not a good means to exhibit the difference. Especially, SiHa, Hela, HT-29 and MDCK are the cell derived from different tissue. Therefore, it is a better way to split the graph figure into five separate graph figure. And TTest should be made between the control group and the treatment group in Figure 1A.

3. Why there are two blot band of CA IX in this figure. If these are isoform of CA IX, please refer it in the discussion or introduction. Even if the phosphorylation assay of Thr443 is a negative result, please put this part in the supplemental figure in Figure 2A.

4. Why the fluorescence intensity is decreased at the concentration 40 mM. And there is no comparison among each group. Please calculate the TTEST value in Figure 2C.

5. Please use different color to indicat the signal from DAPI and the signal from HIF-1#. For example, you could use blue color standing for DAPI and Green color standing for HIF-1#, then merge these two figure. It will be very clear to
show HIF-1# was located in nucleus or not in Figure 3B.

6. why author does not do the CHIP assay to check the binding ability to Glut1 at the same times in Figure 3C.

7. An Ig G control should be set up for the experiment in figure 4B.

8. tumorsphere growth is a good way to check the potential of tumorigenesis. But the sphere size are not supposed the same. So it will be more objective to show more sphere in each figure. Except sphere size, sphere number is also an element to reflect the tumorigenesis. So the author should also compare the sphere number change pattern in each group in Figure 5A.

9. It can not be concluded that the decreased viability is caused by the carnosine. It is possible that the hypoxia condition lead to the decreased viability. Therefore, delete the data from normoxia, and normalize all the data to 0 mM in Figure 5C.

10. The error bar overlapped between Ctrl and Carn treatment group. And there is no statistic comparison in these two group. So it is obscure whether there is significant difference between these two group in Figure 6A, 6B.

11. The English of your manuscript must be improved before resubmission.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.