Reviewer's report

Title: Mitochondria-targeted chromanol inhibits breast cancer cell energy metabolism and promotes cell death

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Reviewer: Yaping Tu

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In a manuscript entitled “Mitochondria-targeted chromanol inhibits breast cancer cell energy metabolism and promotes cell death,” authors Kalyanaraman et al. evaluated selective anti-tumor effects of mitochondria-targeted chromanol (Mito-ChM) on breast cancer cells in vitro and in vivo and also explored the possible underlying mechanisms.

Findings were: (1) Mito-ChM had anti-proliferative effects and cytotoxicity in eight breast cancer cells but little effects on non-cancerous MCF-10A cells, (2) Mito-ChM selectively accumulated in tumor tissue and inhibited tumor growth in a xenograft model of human breast cancer, (3) Mito-ChM caused prolonged inhibition of ATP-linked oxygen consumption rate and selectively depleted intracellular ATP in breast cancer cells, but not in non-cancerous cells, (4) inhibition of glycolysis augmented the inhibitory effects of Mito-ChM.

Overall, this study includes a significant amount of work and fits nicely within the scope of the journal. The data are generally clean and the results are of significant value to the field of cancer research. However, I also raised some major concerns about the paper, which needs to be addressed in the revision.

Major Compulsory Revisions

1. Fig. 1 and Supplemental Figs. 2 and 3 showed that EC50 for MCF-7 cells was 20 uM and no toxicity and cell death were seen after 4 h treatment with 4 uM Mito-ChM. However, data shown in Fig.2 indicated that 4h treatment with 3 uM Mito-ChM was sufficient to induce 75% inhibition of colony formation of MCF-7 cells. Why?

2. Fig.4 and Fig.5 showed that Mito-ChM could accumulate in both cancer cells and non-cancerous cells, and also inhibited ATP-linked oxygen consumption rate in these cells. As expected, there was rapid depletion of intracellular ATP in MCF-7 and MDA-MB-231 cells. However, little depletion of ATP was seen in MCF-10A.

   a) It would be helpful if the authors can comment on the possible mechanism(s) for such difference since ATP depletion is critical for the inhibitory effects of Mito-ChM (Is this true? Any other evidence to support it?).

   b) Is this a general observation in other non-cancerous cells? The authors should
strongly consider additional studies using more non-noncancerous cells.

Minor Essential Revisions:

1. Abstract section: Lane 11, “Mito-ChM …. inhibited intracellular ATP” is not correct. May change it to “depleted”
2. Abstract section: Lane 17, Mito-ChM did not cause tumor regression. It inhibit tumor growth.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests