Reviewer’s report

Title: Neutral sphingomyelinase 2 modulates cytotoxic effects of protopanaxadiol on different human cancer cells

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Reviewer: William Jia

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The novel findings of this study are that PPD depletes neutral sphingomyelin in the membrane of cancer cells. It also increased production of ceramides. The authors have also showed that PPD can cause cancer cell death and enhance the cytotoxicity of chemotherapeutics. Finally, they showed that treatment with PPD on K562 tumour model had equal tumour inhibitory effect as cisplatin but less toxicity. But those have been reported previously by many papers.

Comments

1) Finding PPD depletes neutral sphingomyelin from the cell membrane is important. But the evidence is only by fluorescent image and the authors did not show the sphingomyelin is raft associated. Centrifugation separation of lipid rafts and measure the sphingomyelin content in the rafts quantitatively will be more convincing.

2) Although increase of ceramides was seen at high concentration of PPD (50uM), I am not convinced that is the mechanism of PPD caused cytotoxicity since the toxicity by PPD was evident at 25uM at 24hr when no significant increase of ceramides were seen at 16hr. In addition, knockdown or inhibition of sphingomyelinase by siRNA or GW4869 (an inhibitor) did not really abolish the effect of PPD (only 5-10%).

3) The authors claim that M#CD significantly enhanced PPD’s cytotoxicity (Fig 4). I cannot agree that point. Based on Fig.4A, 0.5mM of M#CD alone has 30% inhibition on K562 cells and 20% on HT29 cells. 25uM PPD alone had 60% inhibition on both cells. But 0.5mM of M#CD and 25uM PPD together still only had 60% cell killing. Therefore, it is clearly that PPD and M#CD at those concentrations had the same mechanism of action and 25uM PPD may be more potent than 0.5mM M#CD (that is why only the same degree of inhibition as PPD alone). Similarly, combination of 25uM PPD with 1 mM M#CD did not show any additive effect of the both (actually less additive). My point is supported by Fig.4B, that clearly showed that M#CD at 1mM had almost identical effect on various proteins to that of 25uM PPD, further suggest the two work on the same mechanism at these concentrations. By the way, the authors should give statistical results of those western blots as one result of western blotting is not sufficient to draw the conclusion.

In conclusion, this work has some interesting results. But the authors need to reconsider their interpretation of those results and change their conclusion.
accordingly.

Although there is a response to reviews by the authors, there are neither specific comments regarding scientific contents of the manuscript nor responses by the authors.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

none of above