Reviewer's report

Title: Saikosaponin-d Increases the Radiosensitivity of Hepatocellular Carcinoma Cell line SMMC-7721 by Adjusting the G0/G1 and G2/M Checkpoints of Cell Cycle

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Reviewer: Yi-Jang Lee

Reviewer's report:

The manuscript "Saikosaponin-d Increases the Radiosensitivity of Hepatocellular Carcinoma Cell line SMMC-7721 by Adjusting the G0/G1 and G2/M Checkpoints of Cell Cycle" provided by Wang et al. proposed that a potent anticancer compound extracted from Chinese herb could increase the radiosensitivity of HCC cell line, and refer to the utility of this compound in clinical radiotherapy. However, there are several data and conceptual problem that obstacle the publication of this work.

Major Compulsory Revisions

1. The authors emphasize that "...their recent clinical practice of combining SSd administration with radiation in treating patients with hepatocellular carcinoma revealed that this joint treatment was more effective than either monotherapy alone, indicating a contributory effect of SSd on radiotherapy." Therefore, they would like to investigate the molecular mechanisms of SSd mediated radiosensitive responses. However, the author did not provide the reference or literature for their previous clinical work. Because the in vivo tumor responses and cell line responses to chemo-radiotherapy may be different, the detailed treatment of patients using SSd and radiation should be stated more detail.

2. Although the author claimed that SSd could increase the radiosensitivity of SMMC-7721 HCC cell ine, no survival curves and dose modifying factor (DMF) were provided in this study. Because the survival curve using colony formation assay (or MTT assay) is the most important parameter to determine the radiosensitivity in treated cells, this data need to provide before SSd can be claimed as a radiosensitizer.

3. The author compared the oxia (or Normaxia?) and hypoxia effects when using SSd and radiation treatment on cells. The idea is good, but use of CoCl2 to represent the hypoxia need to be more conserve. Is it possible to know the level of hypoxia using CoCl2? Also, the authors did not show the change of HIF-1a after CoCl2 treatment. This is the most important evidence that CoCl2 can be used for hypoxic mimetic.
4. In Figure 1, 2Gy seemed still cause the growth inhibition of cells in hypoxia, although no significance was pronounced. Please provide the p value of this group.

5. In figure 2A, the base lines (the crosses) varied in each subfigure of flow cytometric analysis for apoptosis. The cell number also seemed varied a lot in different dataset, please solve these problems or discuss it.

6. It is not clear the purpose of using PTX-478 combined with or without radiation. It should be used with SSd+IR under hypoxic condition, based on the authors assumptions.

7. In figure 3A, what is the blue peak of the cell cycle distribution schemes? The author should address it and define it for discussion, particularly combined with figure 2.

8. In figure 4, the lanes of each blot were not line up, therefore it is difficult to compare the difference between these molecules. Additionally, the HIF-1α level should be added in these data. Is there any relationship between the changes of these molecules to cell cycle redistribution?

9. Overall, because the authors used PTX-478 for almost all data to compare to SSd, it is unclear that the point of this paper is the radiosensitivity increased by SSd, or the importance of hypoxia when using SSd. The authors need to clarify this point in manuscript title and throughout the manuscript.

Minor Essential Revisions
1. Is oxia the normoxia? Please be consistent.
2. In figure 4A, it is suggested that the description of each lane should be put on the top of the blot but not in the middle of two dataset.
3. The topic of each result paragraph need to rewrite to reflect the importance and significance of the findings. For example: "SSd Inhibited Cell Growth", but the data included radiation treatment, then the authors should clearly state the combined treatment is important in the topic.
4. How many time of experimental repeats to obtain the statistical analysis results need to provide.

Level of interest: An article of limited interest

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

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

Declaration of competing interests:
1. No
2. No
3. No
4. No