Reviewer's report

Title: Synergistic Anti-proliferation Effects between Gambogic Acid and Docetaxel in Gastrointestinal Cancer Cells

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Reviewer: Nik Soriani Yaacob

Reviewer's report:

Major Compulsory Revisions

No. Section Comments
1. Abstract Decreased mRNA expression reported is only relevant to GA activity and does not provide any indication on the mechanism of Doc and GA combination effect
2. 3.1, Table 1 & Discussion
   Results for IC50 values are not available in Table 1 as stated. So the statements made cannot be verified. How do the IC50 values obtained compare with other reports?
3. 3.1 & Figure 1 Should include some comments on the results obtained with the combination treatment and indicate any statistically significant differences, in the graph (and include in the figure legend)
4. 3.2 (para 1) The combination concentration ratio is said to be the same as that of IC50 values but this cannot be ascertained since the IC50 results were not shown
5. 3.2 (para 1) The authors stated that “… the combination treatment provided an efficient anti-tumor response compared with single drug application as revealed by the dose-response curves…” However, the graphs (Figure 2) seem to indicate increased survival with the combination treatment compared to GA or Doc treatment alone. The unit in y-axis is confusing. Please clarify
6. 3.2 (para 2) If Table 1 is supposed to list the missing IC50 values, then the CI result summary should be in Table 2
7. Figure 3 Why only show the results for MKN-28 cells? What about the other three cells?
8. Figure 4 To include the percentages of cells in the individual quadrants

9. 3.4 &

Figure 5 The expression of the three genes was determined in one cell line only, i.e., BGC 823 (based on the figure legend). It is not clear why this is so. Furthermore, the measurement was done following GA treatment alone only. What’s more important is to determine (with respect to the current study) any modulatory effects on the gene expression resulting from the combined treatment of GA and Doc. In addition, the same determination should also be carried out on the other three cell lines.

10. 3.4 The statement “GA might promote the anti-tumor effect of Doc through inhibition of #tubulin III, tau and survivin expression” is overstated since the gene expression was determined only in GA-treated cells. As stated above, the experiment is incomplete to make this conclusion

11. 3.4 &

Figure 5 The term “Doc-related genes” is not really appropriate since the genes under study are not unique/specific to Doc action

12. Figure 5 The reviewer assumes that the mRNA expression of each gene is compared to the respective control values (i.e., of untreated cells) but this was not stated in the legend/text.

13. Discussion Some elaboration is needed on previously reported growth inhibitory and apoptotic effects of GA in vitro (eg., Yu et al., 2007) as well as in vivo (for eg. in BGC 823 nude mice xenografts by Liu et al., 2005) and describe the mechanism of action of GA, based on the published reports.

14. Discussion

(p11, para 1) The statement “… while MKN-28 was insensitive to Doc application” does not tally with the results shown (Figure 1B)

15. Discussion

The mechanism of promotion of Doc-induced apoptosis via mRNA expression of related proteins (survivin, #tubulin III and tau) has not been demonstrated. The fact that the researchers only showed the mRNA expression with GA alone does not reflect the conclusions drawn of the synergistic mechanism of GA with Doc

16. Discussion Limitations of the study should also be included

Minor Essential Revisions

No. Section Comments

1. Title The manuscript title should be changed to “Synergistic anti-proliferative effects of gambogic acid with docetaxel in gastrointestinal cancer cell lines”

2. 2.4 Please include a reference and/or software used for the determination of synergism

3. 2.4 For the calculation of combination index in the current study, what assumption was made in terms of the mechanism of actions of both compounds? Mutually exclusive or non-exclusive?
4. 3.4
“the” should be added before “expression” (line 4)

5. Figure 2 Add a short description on what ‘fraction affected’ means in the figure legend

6. Figure 4 To include the percentages of cells in all quadrants

7. Figure 5 State the n value

Discretionary Revisions

No. Section Comments
1. Background (para 2) “widely used taxane used to treat” should be replaced with “widely used taxane for the treatment of”
2. Figure 3 The title could be changed to “Induction of MKN-28 cell death by GA and Doc”
3. Figure 4 The title could be changed to “Induction of apoptotic cell death by GA and Doc”
4. Discussion The authors could include in their discussion, the synergistic activity of GA with 5-fluorouracil observed in BGC 823 cells, reported by Wang et al., in Toxicology, 2009.

Whilst the study has some merit, it is important to be consistent in providing complete results for all cell lines used to demonstrate synergistic mechanism of action of both compounds and to draw proper conclusions.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests