ANSWERING REVIEWERS

Nov 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13959-edited.doc).

Title: Growth inhibition and apoptosis induction by alternol in human pancreatic carcinoma cells

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The manuscript has been improved according to the suggestions of reviewers:

1 The format has been updated;

2 The revision has been made according to the suggestions of the reviewers;

(1) Reviewer 1

Reviewer's comments: "You can not draw the conclusion according the experiment, for it test the functions of alternol only in cell line."

Response: Agree. This is just an in vitro study of cell line, and our results only suggested that alternol may provide an effective regimen for the treatment of pancreatic cancer.

Reviewer's comments: Please supplement picture about: "Alternol has shown a dose- and time-dependent inhibition for the proliferation of the PANC-1 and BxPC3 cells in vitro".

Response: A MTT assay to measure the in vitro proliferation of the PANC-1 and BxPC3 cells may suit this. Unfortunately, as our university is moving to a new campus, this could only be done in our subsequent studies.

Reviewer's comments: The authors enumerate several molecules related to apoptosis and consider these molecules involve mechanism of apoptosis, but the author didn't test which one is the key factor further.

Response: Caspase 3, Bcl-2, p53 and p21 are all known to be involved in the process of apoptosis. Based on our results, such molecules are all involved in alternol induced apoptosis and cell cycle arrest, but we could not pinpoint which one is more important.

(2) Reviewer 2

Reviewer's comments: The present data suggest that alternol has effect on cell cycle arrest at S phase. Does the agent rather promote G1 to S transition?

Response: This is also possible, However, we could not find evidence to support this.

Reviewer's comments: Page 11. If the alternol activate Caspase 3, then degradation of pro-Caspase 3 should be demonstrated by showing increased fragments.

Response: Theoretically, reduction of pro-Caspase 3 may be demonstrated by increased fragments. However, should degradation of such fragment occurs quickly, we may not catch this increase. We did not notice the fragments on our films. This may be due to the minimum amount of such fragment or rapid degradation of such fragment.

Reviewer's comments: Does the p53 in the pancreatic cell lines mutated? Does an increase in the level of mutant p53 lead to restoration of its function?

Response: Indeed, both PANC-1 and BxPc3 cells may express mutant p53, (see Ref. 1, Barton CM, et al. Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer. Br J Cancer 1991, 64:1076-1082." And an increased level of mutant p53 can lead to restoration of its function (Ref. 2 "Ji Q et al. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. PLoS One. 2009; 4(8):e6816).

Reviewer's comments: The discussion section seems to be redundant, since the authors discussed one by one the steps of cell proliferation and cell cycle that are serially associated as signaling cascade.

Response: We have reduced this content in our revision.

Reviewer's comments: In the results section, description of methods that is redundant in the results section is found in every section.

Response: We have rewritten our results section.

Reviewer's comments: In Page 12, the explanation of JC-1 should be in the method section and is redundant in the discussion section 3

Response: We have moved this part to our method section in the revision.

Reviewer's comments: In Page 13, "the ratio of anti-apoptotic versus pro-apoptotic Bcl-2 proteins" should be read as "the ratio of anti-apoptotic versus pro-apoptotic Bcl-2 family proteins"

Response: We have changed this in our revision.

Reviewer's comments: In the abstract: TdT-mediated dUTP nick end labeling (TUNEL). No need to abbreviate.

Response: We have deleted this abbreviation.

(3) Reviewer 3

Reviewer's comments: Major points 1. The authors used only pancreatic cancer cells for all assays. However, because they used no control of non-malignant cells, it is unclear whether the effect of alternol is cancer cell-specific.

Response: We have initially tried to find normal pancreas cell lines but had failed. Nevertheless, we did test the effect of alternol on human umbilical vein endothelial cells (HUVEC) and found that it had much less effect on such cells compared with pancreatic cancer cells.

Reviewer's comments: 2. The mechanism of anticancer effect of alternol seems to be somewhat different in two pancreatic cancer cells, which should be discussed more in the discussion.

Response: This may be attributed to the cells themselves or the condition of the cells. It will be worthy to identify the particular signaling pathways involved in such cells.

Reviewer's comments: Minor point 1. Fig.1 should show the results of statistical analysis.

Response: We have modified Fig. 1 in our revision.

Reviewer's comments: 2. All the figures show the results from two cell lines independently. However, the data could be present in a more concise way.

Response: We have modified our figures to be more concise.

Reviewer's comments: 3. In fig.5, the percentages of red and green shown in the graph seem to be inconsistent with the figures of flow cytometry.

Response: The flow cytometry figures are just one representative of our results, while the percentages of red and green signals are mean of 3 experiments.

Reviewer's comments: 4. In fig. 6, it is not clear what the y-axis represent in the graphs. Moreover, Western blotting should show the results from all the concentrations of alternol.

Response: We have added description for the y-axis and have shown all the conc. of alternol in this figure.

(4) Reviewer 4

Reviewer's comments: Clear manuscript, would have expected though a more comprehensive discussion. Please review this section of your submission.

Response: Thank you very much for your kind suggestion. We have edited this section.

(5) Reviewer 5

Reviewer's comments: 1. Statistical analysis of the results show that only the effect of alternol on apoptosis has a statistically significant strength ($p<0.01$), while the rest of the results depict a trend.

Response: Agree, We have performed statistical analysis for all of our results. Since alternol has shown significant differences at all conc. compared with the control, Figure 1 may look a bit complicated in that we did not add * to the Fig. As for Fig. 2 (showing the ratio of G1, S and G2/M) and Fig. 5 (showing the ratio of green to red), we were also afraid of confusion so we did not add * to the Figure. We have modified this in our revision.

Reviewer's comments: 2. The manuscript needs linguistic improvement.

Response: We have tried our best to improve the language.

Reviewer's comments: The design of the study has no systematic biases, the results, however, depict statistical strength only regarding apoptosis and show a trend towards antitumoral effect of alternol regarding other properties. This issue in combination with the fact that this study is an in vitro investigation should be strongly referred in the manuscript in order to be accepted in your journal.

Response: Agree. We have stressed the in vitro nature of our study in our revision and emphasized the need for further investigation.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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