Author’s response to reviews

Title: Dihydroisotanshinone I combined with radiation inhibits the migration ability of prostate cancer cells through DNA damage and CCL2 pathway

Authors:

I-Yun Lee (ynalee@cgmh.org.tw)
Yin-Yin Lin (lin645@cgmh.org.tw)
Yao-Hsu Yang (r95841012@cgmh.org.tw)
Yu-Shin Lin (yohimba@cgmh.org.tw)
Chun-Liang Lin (linchunliang@cgmh.org.tw)
Wei-Yu Lin (lwy0912@cgmh.org.tw)
Yu-Ching Cheng (a8890927@gmail.com)
Li-Hsin Shu (pipi19880706@yahoo.com.tw)
Ching-Yuan Wu (smbepigwu77@gmail.com)

Version: 1 Date: 25 Aug 2017

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Response to the reviewers’ comments

Here are our responses to improve the manuscript, in light of the reviewer’s comments. (Point-by-point).

Reviewer reports:

Tao Che, Ph.D (Reviewer 1): In this manuscript, the authors investigated the anti-cancer effect of DT. The results are clear. But given that the authors have already published a very similar work early this year (Wu CY et al, Oncotarget, 2017), the work here loses lots of novelty. Although the authors tried to combine it with radiation and detected some other responding proteins, there are still several major issues:

1. Based on Fig.1 and 2, can you say there is no cooperative activity between DT and radiation? Because there is very small difference between "5 uM DT" and "5 uM DT and 5 Gy IR", the small difference seems to be the result of IR.
Response:

We thank and agree the reviewer’s comment. In the Figure 1 and 2, the small difference between "5 μM DT" and "5 μM DT and 5 Gy IR" should be the result of IR. We have rewrite the revised manuscript depending on the reviewer’s comments. (Discussion section, line 13, page 17)

2. As said before, most of the results here have been shown in a previous paper they published and don’t know why they didn’t cite the paper. Although they detected increased expression level of r-H2AX and p-ATM. It doesn't necessarily mean they are involved in DT-induced anti-cancer effect. Is it possible they are just side effects of DT treatment? Blocking this pathway will restore the cell migration?

Response:

We thank the reviewer’s comment. Because the manuscript was prepared before publication of the previous paper[1], we forget to cite this paper. We have cited this paper in the revised manuscript. In our previous paper, we analyzed the whole genomic mRNA expression profile of DU145 cells treated with DMSO or 10 μM DT for 24 h through an mRNA array[1]. Our mRNA array data also identified 24 pathways in DT-treated DU145 cells through GO enrichment analysis based on biological processes, including apoptosis and amino acid biosynthesis. Because these pathways can change the chromatin structure[2, 3], it is possible that these signaling pathways can induce r-H2AX /p-ATM pathway. We have rewrite the revised manuscript depending on the reviewer’s comments. In the other hand, r-H2AX /p-ATM pathway involved many pathways. It is very difficult to block r-H2AX /p-ATM pathway through regulating any one of these pathways. We also found DT and combined treatment with IR (5 μM DT plus 5 Gy IR) significantly inhibited the secretion of CCL2 from the prostate cancer cell (Fig. 3b, d). To validate the critical role of CCL2 in controlling the migration ability of DT-treated prostate cancer cells, we investigated the effects of DT or combined treatment with IR (5 μM DT plus 5 Gy IR) and with or without CCL2 on the migration ability of prostate cancer cells. After adding 5 pg/mL of CCL2 to the conditioned medium, we observed that CCL2 partially rescued the migration ability of DT- or combination-treated PC-3 cells (relative migration: from 30–40% to 60%) (Fig. 3f). Our results suggest that CCL2 is among the cytokines that control the migration ability of DT- or combination-treated prostate cancer cells, and that DT and combined treatment inhibit the migration of prostate cancer cells through their effects on CCL2. (Results section, line 5-13, page 14)

3. The authors didn’t use normal cells as a control? Are these events also occurring in normal cells?

Response:

We thank the reviewer’s comment. In our previous study, we found that the proliferation of IMR-90 cells (normal human lung fibroblasts) was mildly inhibited by 5 μM DT treatment for 24 hours [4]. In addition, we discovered that 5 μM DT inhibits the migration ability of IMR-90 cells (Fig. 3e). The migration ability of IMR-90 cells was mildly inhibited by 5 Gy IR. In the present study, combination treatment with 5 μM DT and 5 Gy IR was also able to inhibit the migration
ability of prostate cancer cells. These data suggest that DT and the combined therapy can inhibit the migration ability of both prostate cancer cells and normal human lung fibroblasts. (Results section, line 14-18, page 14, line 1-3, page 15)

Devarajan Rathish, MBBS (Reviewer 2): Useful paper which needs a re-write.

There needs to be a title page.

Response:

We thank the reviewer’s comment. We have added a title page. (Title section, page 1)

Introduction section should not have results of the present study.

Response:

We thank the reviewer’s comment. We have rewritten the section of introduction. (Background section, line 11-18, page 6)

Methods could be organized for better understanding.

Response:

We thank the reviewer’s comment. We have rewritten the section of Methods. (Methods section, page 7-11)

Results section should not have or repeat methods.

Response:

We thank the reviewer’s comment. We have rewritten the section of results. (Results section, page 12-15)

There are two separate sections on results and discussion. However, it is noted that discussion is present within the results section as well. Needs an edit.

Response:

We thank the reviewer’s comment. We have rewritten the section of results and discussion. (Discussion section, page 17-21)

The phrase "DT and Combination therapy" has been repeated many times. Combination therapy also includes DT. It is unclear especially in the results section.

Response:
We thank the reviewer’s comment. We have rewritten the section of results. (Results section, page 12-15)

Conclusion focuses only on DT whereas, the main objective seems to be on the combination therapy.

Response:

We thank the reviewer’s comment. We have rewritten the section of conclusion. (Discussion section, page 17-21)

The declaration section needs re-ordering and filling of blanks!

Response:

We thank the reviewer’s comment. We have rewritten the section of declaration. (Declaration section, page 23)

Usman Tambunan, Ph.D (Reviewer 3): Manuscript entitled: "Dihydroisotanshinone I combined with radiation inhibits the migration ability of prostate cancer cells through DNA damage and CCL2 pathway" is very well written and has a significant novelty. This manuscript also has quantitative data that was designed and analyzed statistically acceptable.

I recommend that this manuscript be accepted for publication

Response:

We would like to take this opportunity to express our sincere thanks to the reviewer.

Reference:


