Author’s response to reviews

Title: Phenethyl isothiocyanate upregulates death receptors 4 and 5, sensitizes TRAIL-induced apoptosis, and inhibits proliferation in human cancer stem-like cells

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Author’s response to reviews:

Dear Dr. Lin,

We greatly appreciate the helpful comments from the two reviewers. We have carefully reviewed their comments and your suggestions and incorporated them into the current revised manuscript submission. We hope that the revised version will be suitable for publication in BMC Cancer.

For your ready reference, we have detailed our response below to the individual reviewer comments within the context of their critiques.

Please also note that text modifications in response to reviewers’ comments are tracked and highlighted in the revised manuscript.

Sincerely,

Moul Dey
July 3, 2014

Response to Reviewer# 1 (Dr. Sung-Dae Cho)

1. The authors used only one specific cell line, HeLa. They need to use another Cancer cell line to generalize their conclusion.

Response: We share this concern by Reviewers# 1 and 2. According to data reported by Gu et al (Reference #5), HeLa is one of the two cervical cancer cell lines forming spheres among four tested cell lines (HeLa, CaSki, SiHa and C33A). Compared to the other cell line (SiHa), spheres derived from the HeLa cells have cancer stem cell-like nature (ability to differentiate, resistance to cisplatin and doxorubicin, CD44High/CD24Low). As such we do not have access to another known cervical cancer stem cell system to validate our current findings. In addition, Phenethyl isothiocyanate (PEITC) has been
well-documented to inhibit cell growth and induction of apoptosis in many cancer cell lines (Tang NY et al., Cancer research 2011; Gupta P et al., BMC Medicine 2012; Chen P Y et al., Evidence-Based Complementary and Alternative Medicine 2012). Furthermore, our unpublished data also suggested PEITC induce PARP cleavage in colon cancer cell line SW480. Importantly, PEITC also has been shown to inhibit other tumor growth in an animal model (Singh SV et al., J National Cancer Institute 2012). Based on these previous studies, we hypothesize that the tumor-limiting mechanism by PEITC is largely broad-spectrum and not just for cervical tumors, which will form the basis of our future investigation.

2. The title mentioned that PEITC can sensitize TRAIL-induced apoptosis. However, data seems not to represent authors’ description. Figure 3A and 3B showed that the effect of combination therapy of PEITC and TRAIL is less than additive effect. It suggests that PEITC cannot sensitize TRAIL-induced apoptosis.

Response: We noticed the trend of PEITC sensitizing TRAIL-induced apoptosis is not highly striking in cancer stem cells. Cancer stem cells have the nature to repel drugs and resist to apoptosis. With this consideration, we discussed cautiously the synergistic effect of PEITC to TRAIL-induced apoptosis. In figure 3A, 15µM PEITC and TRAIL showed significant higher level of cPARP compared to either Trail or PEITC alone. In figure 3B, only TRAIL, or only PEITC still induced cell apoptosis, but synergistic treatment of PEITC and TRAIL resulted in an increased induction of apoptosis. Further, TRAIL sensitization by PEITC was observed in context of receptor upregulation in Figure 4. Therefore, we are comfortable with the present title. However, if the reviewer/editor decides otherwise, we will respect their judgment and modify the wording in the title.

3. Please, modify experimental designs for Figure 4. Authors'd better use 4 groups as they did it for Figure 3. (control, PEITC treatment only, TRAIL treatment only and combination)

Response: We apologize that we did not articulate clearly the purpose of this experiment and also the figure labeling for 4A are confusing. In this experiment, we focused our investigation on PEITC-mediated enhancement of TRAIL-induced DR4 and DR5 upregulation. Since TRAIL-signaling is well-characterized, we did not attempt to show TRAIL effects on DR4 and DR5 by comparing with a DMSO control alone. Instead our control for this experiment is TRAIL treatment alone as shown in Figure 4B. We compared the combined effect of PEITC+TRAIL with TRAIL alone on each of DR4 and DR5 and observed significant TRAIL sensitization of both receptors by PEITC. We now have clarified this point in the text (page 8, 12, 14).

Additionally we have revised the labeling deficiency on Figure 4A. All our treatments are prepared in DMSO, we recognize it is not necessary to designate A-i and A-iii as DMSO. We now have changed the labelling to match Figure 4B. A revised tracked version for Figure4 is uploaded with our revision submission and if accepted we will remove the tracking at a later stage.
4. In Result section, authors performed only detection of PARP cleavage, but they described caspase-dependent pathway. If authors mentioned it like this, they should detect caspase activity and use caspase inhibitors.

Response: We have modified the text as suggested by the reviewer (page 11).

Response to Reviewer# 2 (Dr. Huey-Jen Lin)

1. The whole study was built upon the findings from one cervical cancer cell line known as HeLa which might be somewhat insufficient. As the following cervical cancer cell lines are commercially available from ATCC, it might be feasible for authors to include another cell line to provide an un-biased study.a. Ca-Ski, ATCC CRL-1550, b. DoTc2,-4510 ATCC CRL-7920, c. SiHa, ATCC HTB-35 d. C-33-A, ATCC HTB-31.

Response: We appreciated this comment and will incorporate these cell lines into our future study. DoTc2,-4510 has not been tested for sphere-forming ability, and we would be very interested to find another cervical cancer cell line that can be used for our future cancer stem cell study.

Also we would like to note that according to data reported by Gu et al (Reference #5), HeLa is one of the two cervical cancer cell lines forming spheres among four tested cell lines (HeLa, CaSki, SiHa and C33A). Compared to the other cell line (SiHa), spheres derived from the HeLa cells have cancer stem cell-like nature (ability to differentiate, resistance to cisplatin and doxorubicin, CD44High/CD24Low). As such we do not have access to another known cervical cancer stem cell system to validate our current findings. In addition, Phenethyl isothiocyanate (PEITC) has been well-documented to inhibit cell growth and induction of apoptosis in many cancer cell lines (Tang NY et al., Cancer research 2011; Gupta P et al., BMC Medicine 2012; Chen P Y et al., Evidence-Based Complementary and Alternative Medicine 2012). Furthermore, our unpublished data also suggested PEITC induce PARP cleavage in colon cancer cell line SW480. Importantly, PEITC also has been shown to inhibit other tumor growth in an animal model (Singh SV et al., J National Cancer Institute 2012). Based on these previous studies, we hypothesize that the tumor-limiting mechanism by PEITC is largely broad-spectrum and not just for cervical tumors, which will form the basis of our future investigation.

2. A lack of normal control is another concern. Hence, commercially available non-malignant cervical epithelial cells (from normal individuals) are recommended to be involved with this study, for revealing the background cytotoxicity effect from PEITC and from TRAIL on normal cervical cells.

Response: We agree that this is a very good suggestion. We have searched current cervical cancer stem cell literature and have not found a good normal cell line that has been used in a similar research. We hope in the future we can test more natural compounds in our current HeLa cancer stem cell system along with the normal cells.
3. Biomarkers for identifying cancer stem-like cell remains rather inconclusive and each marker might enrich a distinct subset of cancer stem-like cell population. A recent publication reported in Oncotarget. 2013 Dec;4(12):2462-75 entitled: “High aldehyde dehydrogenase activity identifies cancer stem cells in human cervical cancer” demonstrated that a subpopulation of cervical cancer cells retain high aldehyde dehydrogenase (ALDH) activity that characterizes a subpopulation of cells with cancer stem-like cell (CSC) properties. Therefore, authors are encouraged to substantiate their findings in the ALDH-enriched CSC subpopulation.

Response: As extensive research efforts have been invested in this area, more features of the cancer stem cell will be discovered. Our PEITC and cancer stem cell project completed prior to this referred manuscript published, so we did not get a chance to incorporate the ALDH characterization to this manuscript. We hope in the future we can get a research fund that enables us discover the mechanisms that can significantly advance our knowledge of PEITC.

4. As shown in Figure 4B, elevated expression of DR5 was rather insignificant, after a combined treatment with PEITC and TRAIL. Authors might weaken their tone and further provide alternative explanations.

Response: We have modified the text as suggested by the reviewer (page 3, 8: line #151, 12, 14).