Author's response to reviews

Title: Gamma-tocotrienol treatment increased peroxiredoxin-4 expression in HepG2 liver cancer cell line

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Version: 3 Date: 5 December 2014

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RESPONSE TO THE REVIEWER CONCERNS FOR THE REVISED MANUSCRIPT – Gamma-tocotrienol treatment increased peroxiredoxin-4 expression in HepG2 liver cancer cell line

Herein, I would like to respond on reviewers concern as stated below:

Reviewer: Hiromi Sato
Reviewer's report:

Major Compulsory Revisions

1. Authors stated in conclusion that Prxs4 might trigger or induce the suppression of proliferation HepG2 cells, while it was only shown to be decreased at mRNA expression level after GTT treatment in HepG2 cells in validation experiment. Why it hadn't been shown as change of protein level? Authors also referred its half life in discussion. It was only factor which authors found to be changed significantly after GTT treatment by 2-DE followed MALDI-TOF/MS analysis. Therefore detection of protein level, unavoidable data like mRNA level as validation, should be added with quantitative and reproducible data.

Response:

We believe that perhaps the reviewer was unclear about our results. Our 2DE results showed that Prx4 was increased in expression following GTT treatment (at the protein level). However, there was no correlation with RNA expression as RTPCR results showed that the RNA level for Prx4 was downregulated with GTT treatment. This suggested that the regulation of Prx4 expression was not at the mRNA level. In contrast to the increased expression of Prx4 protein in HepG2 cells after GTT treatment, Prx4 messenger ribonucleic acid (mRNA) was apparently downregulated. It has been repeatedly demonstrated that mRNA levels do not always correlate with protein

"Quantitative real-time PCR was performed to assess the mRNA expression of Prx4. The RT-PCR result showed that the mRNA expression of Prx4 did not correspond with its protein abundance as observed in 2DE. This suggested that Prx4 was not regulated at the mRNA level. In contrast to the increased expression of Prx4 protein in HepG2 cells after GTT treatment, Prx4 messenger ribonucleic acid (mRNA) was apparently downregulated. It has been repeatedly demonstrated that mRNA levels do not always correlate with protein"
expression levels such as that observed in human liver tissue [41] and capsaicin-treated HepG2 cells [42]. Both studies emphasized the probabilities of the involvement of posttranscriptional or posttranslational modifications and also unknown regulatory mechanisms and signalling. An example of complex regulatory mechanisms in the expression of Drosophila Peroxiredoxin I (dPrx I) where the existence of two alternative 5’UTRs (in the mRNA transcript of dPrx I lead to identical coding sequences: namely Ia and Ib [43]. Ia translation is enhanced in steady-state cells while Ib translation is increased in cells under oxidative stress. If this kind of complexity is regulated by the DNA of a mere insect, higher organisms including humans is expected to have a more elaborate and complex cellular and DNA regulatory mechanisms that are still unknown. However, with presently available data, it is not possible to postulate a definite explanation.”

We would like to clarify that PRX4 was NOT the only factor that we found to have changed with GTT treatment. A total of five protein spots were shown to have significantly changed in abundance in our 2DE analysis (two increased and three decreased). Prx4 was however, the only protein that we were able to identify by tandem MS. The remaining four were not successfully identified.

2. In supplement, Table 2 should be revised. It cannot be accepted as scientific one. If data would be shown as a Table, it needed to be quantitative value.

Response:
We disagree. The values shown were indeed quantitative. Please refer to table legend shown

“The symbol “∞” refers to proteins that were detected in only one the samples. It was therefore clearly not possible to put a numerical value when referring to its abundance.”

Minor Essential Revisions

3. Prks seems to be involved with endoplasmic reticulum stress (ER stress) because it is localized there. Because ER stress is one of the trigger of apoptosis in cancer cells after anti-tumor agents, it seems better to add some reference in discussion.

Response:
Noted.
Reviewer: Sara Ladu

Reviewer's report:
1. Major Compulsory Revisions: nothing to say
2. Minor Essential Revisions: the authors have to control all manuscript because I found the wrong use of a term and a lot spelling mistakes.

Response:
We have edited the manuscript for language.

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

Response:
We have edited the manuscript for language.

Thank you

Best regards

DR ZAKIAH JUBRI
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