Reviewer's report

**Title:** The isothiocyanate class of bioactive nutrients covalently inhibit the MEKK1 protein kinase

**Version:** 1  **Date:** 31 July 2007

**Reviewer:** Ann M Winter-Vann

**Reviewer's report:**

General

Overall, I found this paper to be based on very sound science. The experiments were well controlled and the results were clear. The biochemistry was very solid. I agree that the ITC compounds they use do inhibit MEKK1 activity through a modification at cysteine 1238. I think this paper certainly deserves to be published in BMC Cancer.

I do not have any major compulsory revisions or minor essential revisions. I do however have several discretionary revisions that I would like to see included in a revision.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

None

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

None

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Discretionary Revisions (which the author can choose to ignore)

1. In figure 8, the authors show that PEITC dose-dependently inhibits the phosphorylation of SAPK but not of Erk1/2. However, MEKK1 does signal to Erk1/2. Presumably in 400mM sorbitol, other MAK3Ks are signaling to MEK1 and Erk1/2 and this signaling is not disrupted by PEITC. What about other conditions such as EGF stimulation, cold, cytoskeletal stress? I also find that 400mM sorbitol is quite high; I’d like to see this experiment repeated at 200 or 300mM sorbitol (in fact, the experiments in figure 7 were done at 300mM sorbitol).

2. In the discussion, the authors mention that dietary plasma levels of PEITC are often around 2µM, but they don’t see inhibition of MEKK1 until [PEITC] reaches 12.5µM. I would like to see a more thorough discussion of this disparity; have similar differences been seen with other dietary compounds that have demonstrable activity in vivo? In LnCAP cells, do you see an inhibition of MEKK1...
activity with lower doses of PEITC that are maintained over longer periods of time (as presumably a dietary compound would be)?

3. The authors spend a lot of time discussing whether this modification is reversible or stable, and base their conclusion that this is a stable modification on the observation that the modification is not removed by SDS-PAGE. I agree that this is a very important point in thinking about the ramifications of MEKK1 inhibition by ITCs. I would like to see an experiment that demonstrates the stability of this modification. Although it was not mentioned in the results section, Figure 5 suggests that the biotin-ITC does not compete off the PEITC label over the short term. This experiment could be carried out for a longer period of time, perhaps with a higher bio-ITC concentration, to demonstrate that the PEITC label is not competed away by bio-ITC.

4. Finally, there is no mention of a possible fate for the covalently-modified MEKK1. Might it be turned over more rapidly? Or, if modified MEKK1 is stable, could it be acting as a dominant-negative by scaffolding the downstream proteins in a pathway that cannot be activated? I would be interested to hear the authors' opinions.

**What next?:** Accept after discretionary revisions

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.