Reviewer’s report

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

Version: 1 Date: 27 July 2007

Reviewer: Sanjay K. Srivastava

Reviewer’s report:

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

Isothiocyanates (ITC) are naturally occurring agents shown to be effective against various malignancies. Isothiocyanates exert anticancer activity by inducing phase II detoxification enzymes and/or through cell cycle arrest and apoptosis in tumor cells. Diverse signaling mechanisms have been attributed to the growth inhibitory effects of ITCs in cancer cells. ITCs are highly electrophillic compounds and capable of reacting with sulfhydryl groups on amino acids. In the current study, Cross et al demonstrated through the series of experiments that phenethyl isothiocyanate (PEITC) covalently modifies cysteine residue on MEKK1 resulting in the inhibition of its kinase activity. MEKK1 is the upstream regulator of JNK signaling pathway. The authors further showed that PEITC also inhibit the activation of JNK. Although, present study deals with an important issue of protein modification by ITC, there are several concerns which need to be addressed.

Concerns:

1. The concentration of PEITC used in some of the major experiments in the current study is way high (12.5-500µM). These concentrations cannot be achieved clinically. Previous studies have established the IC50 of PEITC in the range of 5-20 µM in different cancer cell lines. Moreover, IC50 of PEITC in LNCaP prostate cancer cells is reported to be around 15µM. Even though, high concentration of PEITC such as 100 µM in the present study can inhibit MEKK1, this high concentration would kill all the cells and would be very toxic to the normal cells in the in vivo setting.

2. Authors have shown that PEITC inhibits JNK activation by MEKK1 inhibition is in contrast with the published reports. Other investigators have demonstrated that PEITC induces apoptosis in cancer cells including prostate by activating JNK pathway and genetically or pharmacologically blocking JNK activation protect the cells against PEITC induced apoptosis. These issues need to be addressed.
3. In the method section, authors proposed to use LNCaP, HeLa and CV-1 cells but in the result section and Figure Legends, mostly the term “Cells” is used. Legends to figures are very confusing and should be rewritten.

4. For clarity purpose, figures should be labeled with appropriate concentrations instead of labeling with 1, 2, 3---or .

5. The word “in vivo” is generally used for studies carried out in animals rather than in cells.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory 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’