Reviewer's report

**Title:** Signal transduction mechanisms involved in S100A4-induced activation of the transcription factor NF-kappaB

**Version:** 1  **Date:** 16 December 2009

**Reviewer:** Charlotta Enerback

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Major concerns:

1. The data presented are obtained from experiments using one specific cell line, an osteosarcoma cell line. Whether the conclusions can be transferred to other cell lines or other types of cancer is not clear. At least, I believe that this limitation should be emphasized. Moreover, the reason for using this specific cell line is not obvious.

2. How was the doses selected for the inhibitors used in Figure 1? I assume the doses used were reportedly efficient to inhibit the relevant pathways in other cell systems, but the inhibitory effect in this specific cell line is not demonstrated. In contrast, the authors used dominant negative MEKK1 to block the MEKK1 mediated upregulation of the NFkB pathway. In this case, the functionality of the construct is presented. The authors do acknowledge this fact by stating in the discussion section page 15, that absence of effect of certain inhibitors may be explained by lack of an optimal experimental setting. Of course, the data would be strengthened by proving at least that H-/ or staurosporine do block the Ser/Thr kinase pathway in this cell line as intended. In addition, is it known whether H-/ or staurosporine have a suppressive effect on NFkB by itself?

Minor concerns:

1. Abstract: In Methods, the specific cell line used should be stated, instead of "cells"

2. Abstract: Conclusions: I am not convinced that the data presented explains how S100A4 promotes metastasis; perhaps this sentence should be rephrased.

3. Why was 2uM S100A4 used, how was this concentration selected?

4. It would be valuable for the readers if the antibodies used could be more specified, there are increasing numbers of antibodies raised against different parts of the proteins, such as many different anti-RAGE.

5. Although the journal have no restrictions in length, I think the methods section, covering 5 pages, could be shortened to get a better balance (results and discussion are 6 pages).

6. On page 11 in results, the authors claim that "Ser/Thr kinases are central players in S100A4-induced NFkB activation" I think this statement comes too early. The data presented do imply these kinases to be important. Next, the authors refer to the fact that target genes are influenced "as expected". Rather, I
think this finding supports the notion that these kinases are important.

7. In figure 1D and 1F, there might be a reduced p-IkBα expression using the highest concentration.

8. In methods, several colon cancer cell lines are mentioned, but I do not appear in the figures.

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.