Author’s response to reviews

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

Authors:

Ida Grotterod (ida.grotterod@rr-research.no)
Gunhild M Mælundsma (gunhild.mari.malandsmo@rr-research.no)
Kjetil Boye (kjetil.boy@rr-research.no)

Version: 2 Date: 22 March 2010

Author’s response to reviews: see over
Dear Editor,

We sincerely appreciate the reviewers’ comments. We have taken the comments into account, and have revised our manuscript as itemized below.

Reviewer 1:
Dr Schneider raises the question that H-7 and/or staurosporine may affect IKK function independent of their kinase-inhibitory activities, such as through induction of apoptosis leading to cleavage of IKKγ. Although it can not be excluded that H-7 and staurosporine partly inhibit S100A4-induced NF-κB activation through other mechanisms, Fig. 5B shows that both inhibitors inhibit IKK kinase activity in a kinase assay using immunoprecipitated IKK complex and recombinant IκBα. Importantly, in these experiments the cells are not incubated with H-7 and staurosporine before cell lysis, and thus no H-7/staurosporine-mediated cell death could occur. This observation is in accordance with published data where staurosporine was identified as an inhibitor of IKKβ (Peet GW, J Biol Chem, 1999). Furthermore, no cleavage of IKKγ was observed after treatment with H-7 or staurosporine, whereas cells induced to undergo apoptosis with IFN-γ displayed marked IKKγ cleavage (figure below).
Further points:

1. Total IκBα levels for all inhibitors are provided in the figure below, and for H-7 and staurosporine also in Fig. 2 in the revised manuscript. Several of the inhibitors used in our study led to reduced total IκBα levels at high concentrations. IκBα degradation and transcription is regulated by a number of signal transduction pathways (Viatour P, Trends Biochem Sci, 2005), and it is therefore not surprising that broad-spectrum inhibitors affect total IκBα levels. Since the focus of this work has been the signal transduction mechanisms upstream of IκBα phosphorylation, we believe that it would be more confusing than clarifying to provide the readers with total IκBα levels for the inhibitors that do not affect S100A4-mediated IκBα phosphorylation. Total IκBα levels after treatment with H-7 and staurosporine are included in Fig. 2 and in the Results section (page 11) in the revised manuscript, and the results are discussed in page 15 and 16.
2. We agree with the reviewer’s comment. This has been included in the revised manuscript (page 10 and 16).
3. Statistics have been included in the revised manuscript (Fig. 3 and 4 and page 10).
4. S100A4-induced expression of ephrin-A1 and optineurin is also inhibited by staurosporine, and these data have been included in Fig. 4C and 4D and in page 11.

Reviewer 2 raised no major or minor concerns.

Reviewer 3:
Major concerns:
1. The reason for using the II-11b cell line is that we have previously shown that S100A4 expression promotes metastasis using this cell system in an experimental animal model, and the effects of S100A4 (both intracellular and extracellular) are well described in this cell system (Mælandsmo GM, Cancer Res, 1996; Bjornland K, Cancer Res, 1999; Pedersen KB, BMC Cancer, 2004; Boye K, Int J Cancer, 2008).

We have confirmed our results in another cell line, and these data are incorporated in the revised manuscript in Fig 3C and page 11. Further confirming our results, ephrin-A1 and optineurin expression is also induced by S100A4 in this cell line (data not shown).

2. The doses of the different inhibitors were based on published data in other cell systems and on recommendations from the manufacturers. For most inhibitors, a relatively wide dose range was employed,
to increase the probability of effect in our experiments. However, we can not exclude that the experimental conditions for certain inhibitors were suboptimal, and this fact is discussed on page 15, as commented by the reviewer. We have verified that staurosporine is able to inhibit Ser/Thr kinases in II-11b cells, and this is shown for TPA-stimulated phosphorylation of MARCKS at Ser152/156 (see figure below).

Moreover, H-7 and staurosporine have no inhibitory effect on NF-κB by itself, as shown in the figure below.

Minor concerns:
1. The Methods section in the abstract has been changed according to the reviewer’s comment (page 2).
2. We agree with the reviewer’s comment. This sentence has been rephrased (page 3).
3. Dose-response experiments using II-11b cells have previously demonstrated that 2 µM S100A4 is the optimal concentration for inducing NF-κB activity (Boye K, Int J Cancer, 2008). Therefore, this concentration was chosen in the present study.
4. All antibodies have been specified in the Methods section in the revised version of the manuscript (page 6).
5. The Methods section has been shortened in the revised manuscript (pages 6-10).
6. We agree with the reviewer’s comment and the manuscript has been revised accordingly (page 11).
7. We agree with the reviewer’s comment. This has been included in the revised manuscript (page 10 and 16).
8. The colon cancer cell lines mentioned in the Methods section appear in Figure 6D in the original version of the manuscript.

Hoping the manuscript will be accepted for publication in BMC Cancer, I am looking forward to receiving your decision.

Sincerely,

Kjetil Boye, MD PhD
Department of Tumor Biology
The Norwegian Radium Hospital
Oslo University Hospital
Montebello
0310 Oslo
Norway
E-mail: kjetil.boye@rr-research.no