Author's response to reviews

Title: The fatty acid binding protein 7 (FABP7) is involved in proliferation and invasion of melanoma cells

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Version: 4 Date: 10 July 2008

Author's response to reviews: see over
Dear Editor

Oslo, 10.07.08

Please find enclosed a revised manuscript by Slipicevic et al. entitled ”The fatty acid-binding protein 7 (FABP7) is regulated independently by PKC and The MAPKK/ERK pathway and is involved in proliferation and invasion of melanoma cells”

We have modified the paper according to the editor and reviewer’s comments and we feel that this clearly has improved the quality of the paper.

Reviewer Yu Liang

We, as the reviewer pointed out, acknowledge the fact that different expression levels of FABP7 could have different effect in the cells which also could explain the observed differences in FABP7 expression between nevi and melanoma. We have included this comment in the discussion in the revised manuscript. However we did not observe differences in the expression levels of FABP7 after treatment with PMA and/or PD98059 which could explain a role in anchorage-independent survival.

1. In our previous publication, cells were grown in suspension for 72 hrs. In that study the main goal was to study the effect of PMA and PD98059 on anchorage-independent survival. Our results showed that the degree of anoikis was most profound after 72 hrs. Based on these results, it is reasonable to assume that regulation of proteins involved in this process occurred on an earlier time point. In the present study we were interested in identifying genes that could possibly be involved in anchorage-independent survival and therefore decided to perform the Affymetrix study on cells treated with PMA and PD98059 for 24 hrs. The gene expression can be a rapid process and therefore we anticipated that the effect obtained after 72 hrs would be a secondary effect and not actually involved in anoikis induction/prevention.

2. We agree with the reviewer that the duration of the treatment could potentially have different effect on FABP7 expression. For this reason we have performed a time course experiment as suggested. Since PMA and PD98059 treatment have similar effect on FABP7 expression in monolayer and suspension grown cells, we chose to perform the experiment in monolayer. We observed that PMA treatment led to down-regulation of FABP7 mRNA and protein after 12 hrs. This effect was sustained for up to 72 hrs. PD98059 treatment led to down-regulation of FABP7 mRNA after 24 hrs, while a slight down-regulation of the FABP7 protein was observed after 12 hrs. Although PD98059 had a weaker effect on mRNA and protein than PMA, the effect was still sustained after 72 hrs. In conclusion, we could not see any variation in FABP7 expression once down regulation was achieved, thus trusting that the chosen 24 hr time point will more likely reflect the correct time frame. A figure demonstrating the results from the time course experiments has been included in the manuscript (Figure 2.).

3. We totally agree with the reviewer that it would be of interest to examine how overexpression of FABP7 would affect proliferation/invasion/apoptosis and have in this regard transiently transfected the low expression LOX cell line with a FABP7 cDNA
Due to the limited time for revision, we have only been able to perform two biological experiments. We could not demonstrate any effect on proliferation and apoptosis and only marginal effect on invasion. Since we have not had time to repeat the experiment at least once more, we do not feel comfortable including the results in the manuscript. However, if the reviewer and editor feel they should be included, we will of course do so.

**Minor essential revisions**

Page 16, second paragraph. “Table 2” has been corrected to “1C”.

**Reviewer Roseline Godbout**

**General Comments:**

1. Based on the presented results our conclusion is that FABP7 is not involved in the apoptosis/anoikis mechanism. Both PKC activation and MEK1 inhibition lead to FABP7 down-regulation but have opposite effect on anoikis. We believe that these effects are independent. PKC activation is most likely affecting additional factors that in turn are regulating survival. FABP7 seem not to have major contribution in this context. In addition to down-regulating FABP7, MEK1 inhibition is probably depriving the cells of important survival factors leading to increased anoikis. We have included this comment in the discussion in the revised manuscript. At this time point we feel it would be difficult to provide a figure which could cover this satisfactory. Further studies should be performed.

2. In agreement with the reviewer’s comment, the title of the paper has been changed. Since we at this point can not provide more details about the regulatory mechanism underlying FABP7 down-regulation after PMA and PD98059 treatment, we chose to remove this from the title.

**Specific Comments:**

In the revised manuscript we have made the following changes:

1. The sentence pointed out by the reviewer on the page 3. has been removed.

2. Pg. 7 Corrected to 0.5µl

3. Pg. 10 Matrigel assay. We have described the media used as the chemoattractant in detail.

4. Use of the word “activated” on page 11 in “activated FABP7 expression” was inadequate and was deleted as well as in the tables.

5. Pg. 14 We have added WM35 to the list of primary cell lines and included a conclusion in the discussion (page 20)
6 and 7. Pg. 15 Figure 4. (previous Figure 3) In the new figure 4 b and c we have related siFABP7-transfected cells to siRNA control as suggested by the reviewer and chosen to present the data as percentage of siRNA control. Furthermore, error bars have been included. The data suggest that proliferation is reduced by approximately 30% in WM35 cells and 80% in WM239 cells by siRNA knock down of FABP7. The original Figures 3d and e were showing one representative experiment for each cell line and for this reason the error bars were omitted. We have now chosen to present the data as percentage of siRNA control in new figure 4d also here error bars have been included. WM239 cell line is derived from a metastasis and is in general supposed to be more invasive than the primary tumor derived WM35 cell line. Even though the reviewer claims that the difference in the number of invading cells in WM35 knockdown cells is greater than in WM239 cells this difference is not significant. However, it is possible that FABP7 contributes more to invasion in primary melanoma cells while it plays a more important role in proliferation in the later stages.

8. The cells were plated in the matrigel chambers 48 hrs after the transfection. This information is now included in the manuscript pg 10.

Minor essential revisions

All minor essential revisions (1-16) pointed out by the reviewer have been corrected as suggested.

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