Reviewer’s report

**Title:** The fatty acid binding protein 7 (FABP7) is regulated independently by PKC and the MAPK/ERK pathway and is involved in proliferation and invasion of melanoma cells

**Version:** 1  **Date:** 1 May 2008

**Reviewer:** Roseline Godbout

**Reviewer’s report:**

In this paper, Slipicevic et al. demonstrate that FABP7 is downregulated in melanoma cells upon treatment with the PKC activator PMA and the MEK1 inhibitor PD98059. Downregulation of FABP7 in two melanoma cell lines using siRNA results in decreased cell proliferation and invasion but does not affect apoptosis. They also immunostain clinical specimens (149 primary melanomas, 69 metastatic melanomas and 11 benign nevi) with anti-FABP7 and suggest that FABP7 may be associated with tumor progression.

**Major compulsory revisions**

**General Comments:**

(1) There is strong agreement between the microarray, quantitative RT-PCR and western data, clearly indicating that FABP7 expression is down-regulated upon treatment of melanoma spheroids with PMA (PKC activator which activates the RAS-RAF-MEK-MAPK/ERK1/2 pathway, PD98059 (inhibitor of MEK1) and combined drugs. Even though the authors address this apparent discrepancy to some extent in the first two paragraphs of the Discussion, it’s still not clear why PKC activation and MEK1 inhibition should have the same effects on FABP7 expression/cell proliferation/invasion (and opposite effects on anchorage-independent growth). Would it be possible to provide a figure depicting the proposed model for these various observations?

(2) Related to the above issue, the title of the paper is somewhat misleading as the authors do not address the regulatory mechanisms underlying FABP7 downregulation as a consequence of PMA and PD98059 treatment. The paper is primarily a study of the effect of FABP7 downregulation on proliferation and invasion in melanoma cells.

**Specific comments:**

(1) Pg. 3. The incidence of malignant melanoma is rapidly increasing in the western world… Don’t some studies suggest that the incidence has stabilized in recent years?
(2) Pg. 7. Clarify amount of cDNA used for quantitative real-time PCR – does 5 ul diluted 1:10 mean 0.5 ul?

(3) Pg. 10. Matrigel assay. FCS is often left out of the top (inner) chamber. Could more information be provided regarding the self-supplied fibroblast conditioned medium that was used as chemoattractant. Alternatively, a reference describing this approach could be provided.

(4) Could you clarify what you mean by activated FABP7 expression – do you mean upregulated FABP7 expression? Activated implies functional activation as opposed to increased levels.

(5) WM35 is included under primary melanomas in Figure 2. This cell line should be added to the list of primary melanoma cell lines provided on pg. 14 (three primary….not two primary). The conclusion from the cell line study is that there is no clear difference in FABP7 levels in cell lines originating from primary tumor versus metastasis. Shouldn’t these results be included in the discussion as the clinical material suggests a correlation between FABP7 levels and tumor progression?

(6) Pg. 14, Figure 3. Percent down-regulation of FABP7 in siFABP7-transfected cells should be relative to siRNA control not to mock-transfected cells. With this in mind, the decrease in FABP7 levels observed in the WM35 line does not appear very substantial, especially when compared to the decrease observed in the WM239 line. While the proliferation data match the decrease in FABP7 levels observed in the two cell lines, there is a much greater decrease in the number of invading cells in the WM35 knockdown cells compared to the WM239 knockdown cells. This should be addressed.

(7) There are no errors bars in Fig. 3D – error bars are needed. Were the error bars omitted because of too much variation from experiment to experiment? If so, this information needs to be included.

(8) Matrigel assay – cells were allowed to invade for 48 hrs. How soon after transfection were the cells plated in the Matrigel chambers?

Minor essential revisions:
(1) pg. 2, 2nd sentence: use understood instead of clarified.
(2) pg. 2, line 12, use with a cytoplasmic and/or nuclear localization instead of at
(3) pg. 3, line 4, use understood instead of clarified
(4) pg. 4, line 3, use “for” the different FABP proteins
(5) pg. 5, line 4, remove “with rather conflicting results
(6) pg. 5, line 7, use “FABP1 levels decrease with”
(7) pg. 6, line 2 – is it possible to define WM
(8) pg. 6, last sentence – could this be reworded as it is confusing (perhaps remove and addition of PMA at end of sentence?)
(9) pg. 7, line 7, use performed instead of preformed
(10) pg. 8, line 10, use re-stained instead of re-hybridized (hybridization usually
refers to DNA or RNA)
(11) pg. 10, line 11, use invading instead of invaded
(12) pg. 11, line 9, use “at” RT
(13) pg. 13, line 4, has “the” opposite effect
(14) pg. 15, line 5..on invasion using the Matrigel assay
(15) pg. 16, line 12. FABP7
(16) Figure 2B – might one of the gels have been flipped? There is an upward trend in the FABP7 gel and a downward trend in the alpha-tubulin gel

**Level of interest:** An article of importance in its field

**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