Reviewer's report

Title: Odontogenic Ameloblast-Associated Protein (ODAM) inhibits growth and migration of human melanoma cells and elicits PTEN elevation and inactivation of PI3K/AKT signaling

Version: 1 Date: 4 February 2013

Reviewer: Clara Montagut

Reviewer's report:

Comments to the Author:

"Odontogenic Ameloblast-Associated Protein (ODAM) inhibits growth and migration of human melanoma cells and elicits PTEN elevation and inactivation of PI3K/AKT signaling" by Foster et al.

The authors have previously published the role of ODAM in breast cancer tumorogenesis. In the present paper they aim to characterize the tumorogenic role of ODAM in melanoma by using melanoma cell lines stably transfected with ODAM. They find that ODAM transfected cells show a decline in growth rate and motility. No changes in extracellular matrix adhesions are found. They find that ODAM transfected cells have a decrease in AKT and an increase in PTEN.

The paper is clearly written, the question posed by the authors is well defended and the methodology is appropriate. Discussion and conclusions adequately support the data and emphasize the limitations of the work. The findings are relevant to those with closely related research interests.

Specific comments:

Major Compulsory Revisions

1- Figure 3. Since previous studies have shown a correlation between ODAM nuclear expression and aggressiveness of the disease, it would be interesting to assess whether ODAM protein is located in the nucleus or the cytoplasm in ODAM positive cells.

2- Page 14, last line and Figure 3. C8161-ODAM cells do not show a truly significant increase in PTEN protein expression (Fig 3B). It would help if authors could include PTEN mRNA levels for C8161-ODAM cells to support their hypothesis that PTEN is increased in these cells (Fig3C).

3- Figure 4A. Surprisingly, in this western-blot, PTEN basal expression shows an important increase in C8161 parental cells versus C8161-ODAM cells (contrary to results shown in Figure 3B, see previous point). Please discuss.

4- Figure 4B. In text (page 15, line 16), the authors state that p-AKT is not increased after PTEN silencing in A375-ODAM cells; contrary to that statement, figure 4B shows an increase in p-AKT in these cells.
5- Figure 4B. Was it not posible to obtain a better PTEN silencing in A375 cells?
6- It would be a relevant confirmatory experiment to downregulate ODAM in an ODAM-expressing cell line (melanoma cell line if possible) and assess if PTEN is decreased and AKT increased.

7- Discussion. Although the role of ODAM in malignancies is unclear, all previous publications show that nuclear ODAM expression is correlated with advanced disease. The present study supports a less aggressive phenotype for cells expressing ODAM. This should be better discussed.

Minor essential revisions

1- Page 14, line 11. Please add reference that supports that c-Raf is a downstream target of AKT. It would be more interesting to assay p-S6 expression in the western blot.

2- Page 12, last line. It is surprising that ODAM transfected melanoma cells do not modify their extracellular adhesion properties. Please discuss.

**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