Reviewer's report

Title: SNAI1 Expression: A Rare Event In Oral Squamous Cell Carcinoma Associated With Features Of Poor Prognosis

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Reviewer: Amparo Cano

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In the present ms. the authors analyse SNAI1 expression in a series of oral squamous cell carcinomas (OSCC) in primary tumours (n= 46) and nodal metastasis (n=26), and compare to the expression of E-cadherin, FAK and p63. They conclude that SNAI1 expression is a rare event in OSCC associated to poor prognosis, as stated in the title.

The authors previously tested two commercial anti-Snail1 antibodies for specificity and select AF3639 antibody for analysis in the OSCC series. The study is well performed in general terms, and the quality of the IHQ is very high for most studied parameters. The conclusions driven from the study can, nevertheless, be confusing or misleading since the term “rare event” is used to refer to the number of tumour cells positive for SNAI1 expression, and not for positive tumours. Present evidence from different studies support that Snail1 expression should be focal, and likely, transient inside tumours and in this sense it will be a “rare” event in the context of the whole tumour but not when considering a tumor series. This also depends on the “cutoff” for positive staining defined by the authors. Therefore, important clarification in this aspect is required for the present study before publication. The presentation of the statistical analyses is also rather confusing and it is sometimes difficult to follow. Additional controls regarding specificity of AF3639 antibody are also required.

Specific points:

1. Specificity of AF3639 antibody is only shown by Western blot (Supp Fig. 1C). It needs to be tested by IHQ on the placental samples, xenografts and spindle cell tumors as performed for SC10432 antibody. In this sense, the data on SC10432 antibody do not add substantial information to the ms. (it is not used for analysis of the OSCC series) and can be removed.

2. The SNAI1 IHQ staining images with AF3639 antibody should be shown for tumours representative of the different H-score categories used by the authors. Only one case with SNAI1 staining in the stroma is presented in Fig. 1, apart from the two samples with sarcomatoid component (Figs. 4 & 5).

3. The most questioning part of the ms., however, is the definition of the term “rare event”. Indeed the data indicate that 22% (10 out of 46) of primary tumors and 19% of nodal metastasis (5 out of 26) are SNAI1 positive (category 2, >5% tumor cells). In addition, “rare” individual SNAI1 staining (<5%) was detected in 30 patients, that is in 65% cases. This figure could indicate that the majority of
tumors indeed express SNAI1 even if it is detected in few tumor cells. This could be particularly important if expression is mainly detected at invasive or inflammatory areas as the authors indicate. Therefore, expression of SNAI1 can not be considered as a “rare event” in the context of the whole tumor series. This analysis thus requires clarification of the term “rare event” and reconsideration of the ms. title.

4. Some recent studies by other authors have used a threshold of >1% positive tumor cells (Blechschmidt et al. Br J Cancer, 98: 489-95, 2008; Franci et al. PLoS ONE, 4(5): e5595, 2009). The author should discuss their data also in the context of those previous works.

5. Presentation of data in Figs. 4 & 5 is very confusing and not easy to follow. Please, distinguish more clearly the information regarding different markers.

6. Statistical analysis in Table 2 is also confusing and hard to follow. One problem is that the series is biased towards grade 2 tumors (37 out of 46) while they compare G1&G2 vs G3 tumors. Since G2 present invasive areas, this group should be compare to G1. On the other hand, if no significant differences are found in any parameter, Table 2 could be deleted.

7. Recent work by Franci et al (PLoS ONE, 2009) has also shown SNAI1 expression in tumor stroma associated to poor prognosis in colon cancer. This study should also be discussed.

8. Indication that EMT can be a focal and transient event and the participation of different EMT regulators was indicated in Franci et al (2006) as well as in other previous works (i.e, Thiery, Nat Rev Cancer, 2: 442-54, 2002; Huber et al. Curr. Opin. Cell Biol., 17: 548-58, 2005; Peinado et al., Int. J. Dev. Biol., 48: 365-75, 2004) that should also be considered.

Overall recommendation: Major Compulsory Revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

We hold a patent on Snail1 as a diagnostic marker, licensed to a biotech company (Advancell Invitro Cell Technologies). Funding for 18,000 € was obtained in 2004 from that company.

I do not have any other financial or non-financial competing interests in relation to this paper.