Reviewer's report

Title: Resistance to growth inhibition by TGF-beta is associated with a partial loss of Smad signaling in the absence of alterations of Smad protein levels during in vitro progression of HPV16-immortalized human keratinocytes

Version: 1 Date: 19 April 2013

Reviewer: Amy Baldwin

Reviewer's report:

This study is well presented, and the data shown is accurately and clearly described for the reader. Please consider the discretionary revisions below.

Major Compulsory Revisions
None

Minor Essential Revisions
None

Discretionary Revisions

1) The authors discuss that immortalization by HPV16 DNA triggers an increase in Smad4 levels that is maintained in later stages of in vitro progression, although reduced levels of Smad4 have been documented previously in the HPV16-positive SiHa human cervical carcinoma cell line and Smad4 is decreased in 90% of cervical squamous cell carcinomas. Additionally, it is pointed out that there are studies that correlate weak cytoplasmic Smad4 staining and the absence of nuclear Smad4 protein expression with more serious disease outcomes and poor survival in cervical cancer patients.

a. Although an increase in Smad4 levels was shown in this study (Figure 2C), the levels of Smad4 in Normal HKc shown in Figure 1C when compared to Figure 2C seem to be different (when comparing Smad4 levels to the loading control actin levels). It would be helpful if the authors could explain this difference.

b. It appears that overall Smad4 levels do increase in response to immortalization by HPV16 DNA. Although the authors demonstrate that the time course of Smad4 nuclear accumulation was similar throughout their in vitro model system of progression following TGF-# treatment, it would be interesting to see both the nuclear and cytoplasmic levels of Smad4. Similarly, it would be interesting to see the nuclear and cytoplasmic levels of Smad3, as the relative% of nuclear Smad3 appears to be much greater in HKc/HPV16 and HKc/HDR when compared to HKc before TGF-# treatment (although overall Smad3 levels appear to be similar in all lines tested, Figure 2B).

2) It is logical to deduce that low expression of TGFBR1 in the cells used could explain the alterations observed in Smad2 phosphorylation levels, even though the Smad system appears to be at least partially or even mostly intact in
HKc/DR. The authors also show a slight delay in Smad3 nuclear accumulation in HKc/DR compared to HKc and HKc/HPV16, but were Smad3 phosphorylation levels investigated as well?

**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