

## Reviewer's report

**Title:** High risk HPV types 18 and 16 are potent modulators of oral squamous cell carcinoma phenotypes in vitro

**Version:** 1 **Date:** 28 May 2007

**Reviewer:** Maria Lina Tornesello

### Reviewer's report:

#### General

The manuscript by Christensen et al. describes the phenotypic changes of the oral squamous cell carcinoma CAL27 following stable transfection with HPV16 and HPV18 alone or in combination. The results obtained with a number of different assays (morphology, viability, adhesion, proliferation) show alterations of CAL27 cells conferred by HPV18 alone or in combination with HPV16 comparable to those previously described by the same authors in HPV16 transfectant of the same cell line (Kingsley et al, 2006). Although these findings are relevant to the HPV-associated oral cancer pathogenesis, there are too many overlapping issues with the previously published paper, which significantly reduces the relevance of the present paper. Nevertheless, if the Authors can significantly improve the results observed with HPV18, addressing the issues listed below, the manuscript can keep its relevance.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The authors should emphasize the new results obtained with HPV18, mentioning those previously obtained on CAL27 cell line transfected with HPV16 only for comparative purposes.

Considering that HPV types other than HPV16 and 18 have little chance to be associated with cancer of the mouth and pharynx, major differences in biological alterations would be expected transfecting oral cell lines with high risk HPVs other than HPV16/18. A different outcome from transfectants obtained with different high risk HPVs, reflecting the in vivo tumor progression of HPV infection in oral cancers, would further validate the HPV pathogenetic model based on CAL27 oral cell line.

#### Specific points:

1. Title: The title should be changed as follow: "High risk HPV type 18 is a potent modulator ...." Omitting the HPV16 in the title.
2. Abstract: in the Results section is not clear if CAL27 are transient or stable transfectant, please specify. The results must be more focused on HPV18: first describe HPV18 results and than compare to HPV16/18 and HPV16.
3. Background, first sentence: The following sentence must be introduced: ".....according to epidemiological case-control studies (Munoz et al. 2003), 15 high-risk types have been acknowledged ( i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82); 3 types were classified as probable high-risk types (26, 53, and 66); and 12 were classified as low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89).....".
4. Results, Transfection: the authors describe transfection of CAL27 with high risk genotype, it is not clear however which type of transfectant are obtained and used for biological assays: are they stable transfectant, as suggested by the use of G418 selection in M&M? If yes, how many resistant colonies have been obtained with the different constructs? Have the authors obtained and expanded different clones and tested in parallel? or have they used pooled populations? Have been the transfected CAL27 analysed by southern blot to verify the HPV copy number integrated per cell?  
Cell spreading: specify the acronym NS.
5. Legends: Fig. 1: the title of the legend "HPV-negative untransfected cells do not express HPV mRNA" assess a quite obvious observation! The innovative observations described in the figure, instead, should be underlined.

6. Figures: Improve the quality of the figures 2, 4, 5, 6, 7: the x or y legends of all histograms have a grey background.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the 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:** No, the manuscript does not need to be seen by a statistician.