Reviewer's report

Title: Cyclin A1 shows age-related expression in benign tonsils, HPV16-dependent overexpression in HNSCC and predicts lower recurrence rate in HNSCC independently of HPV16

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Reviewer: Max Robinson

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This study examines head and neck SCC for a number of biomarkers, namely HPV DNA, p16, p53 and CyclinA1.

The study includes 81 head and neck SCC and 74 patients with reactive tonsils.

Major compulsory revisions

HPV related SCC of the oropharynx are biologically different to their ‘non-HPV related’ SCC counterparts and are also different from SCC at other sites in the head and neck. The current study includes 63 cases from the oropharynx and 18 cases from other sites. It would be useful to indicate which subsites harboured HPV infection - probably the oropharyngeal cases based on established literature.

Increased Cyclin A1 expression correlates with HPV16 infection (DNA and p16 expression) and improved local control. Perhaps by restricting the study to just the oropharyngeal SCC similar trends would be observed, but this would underscore the clinical utility of the test in this specific disease setting. The inclusion of a small number of cases (n=18) from other subsites (oral cavity, hypopharynx and larynx) potentially detracts from what could be a stronger message: ‘increased Cyclin A1 expression can be used as a marker of loco-regional control in oropharyngeal SCC irrespective of HPV status’?

For the immunohistochemical analysis the majority of the cases were analysed providing full data for the cohort. Where laboratory analysis was restricted by availability of tissue (p53 mutations n=20 and Cyclin A1 methylation n=44) the interpretation of the results are limited by the small sample size.

The immunohistochemical tests were assessed by ‘at least 3 pathologist’. Immunohistochemistry scoring is typified by inter-observer variation and it is not clear how discordant scores were resolved to achieve a ‘consensus’ score for each case.

Whilst the ‘cut off’ for positive staining are justified in the text, it must be acknowledged that these values are essentially empirical. p16 overexpression is widely used in clinical practice and much of the published work is based on a nominal ‘cut off’ of strong nuclear and cytoplasmic staining in >70% of the tumour (Singhi and Westra, 2010), 10% in this study seems rather low.
There are no photomicrographs included to allow the reader to visualise the staining localisation or intensity. This is particularly important for Cyclin A1, which is the novel aspect of this study, as other researchers will not be able to repeat the work if there are no photomicrographs to ‘benchmark’ further studies.

The correlative nature of the study makes the discussion rather speculative and the mechanisms are not clear. The clinical utility of Cyclin A1 requires validation in larger studies and the functional consequences of Cyclin A1 overexpression are unclear.

**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