Author's response to reviews

Title: A Survey of CK8 Expression in Head and Neck Epithelia

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Author's response to reviews: see over
Dear Ashley Manning, dear referees,

Herewith, I would like to reply in a point-to-point fashion on the reviews to our abovementioned manuscript submitted to BMC Cancer for publication.

Firstly, my co-workers and myself thank all persons who participated in reviewing our manuscript and therewith helped to improve considerably its overall quality. We have addressed all points of criticism raised by the three expert referees and hope the manuscript now meets the standards for publication in BMC Cancer. For the sake of simplicity we have performed all changes to the manuscript using Word’s tracking system. Human samples were obtained after informed consent and according to the Helsinki Declaration. All information on ethical issues are now stated in the revised manuscript, including filing number of the ethics approval.

With best regards,

Olivier Gires,

Corresponding author
Referee: Alberto Gasparoni

Major compulsory revisions
1) Lacking hypothesis/es: what was/were the goal/s in doing immunohistochemistry?

Answer: We have shown previously that keratin 8 (K8) is absent from normal squamous epithelium of the head and neck area, while it is highly expressed in malignancies of the same localisation. However, these observations were done in a small cohort of patients and did not subdivide malignancies in sublocalisations. The goal of the present study was to substantially enlarge the patients’ cohort and to subdivide malignancies. The reason to use IHC as a method of choice is that it allows thorough detection of K8 and proper assignment of the staining to different cell types within tumour samples. This issue is now referred to in the Introduction section on page 4 of the revised manuscript.

Minor essential revisions
1) The analysis of CK8 expression in normal epithelia is not clear. There is no statistical analysis, and samples of normal epithelia include a variety of anatomical sites.

Answer: Since all normal mucosa samples were negative for K8 except for laryngeal epithelium and for the case of normal epithelia derived from tumours where some carcinoma cells were detected within the normal sample (see table 1), the statistics are as follows: K8 expression was negative in 100% of normal oropharyngeal epithelium. This is now mentioned in the revised manuscript on page 8. Furthermore, all samples of normal mucosa originated from the oropharynx and larynx as mentioned in table 1 (last row).

2) Results should follow hypothesis/es enunciated in introduction (example: first…)

Answer: We have changed the manuscript accordingly. The overall manuscript’s results structure now adheres to the hypothesis enunciated (see pages 7-9). In order to meet these changes, figure numbers have been changed likewise.

3) How was the weak/strong expression of CK8 evaluated? Were the two investigators calibrated? (CK8high although intuitive, is not defined throughout the article).

Answer: The evaluation of K8 expression levels followed scoring from negative (-), through low (+) and intermediate (++) up to strong expression (+++). This evaluation was described in more detail and illustrated in adequate figures in Gires et al. BBRC 2006. This issue is now implemented in the revised manuscript, including the according reference.

CK8high was explained in more detail as it represented K8 +++ expression (see bottom of page 9).

Discretionary revisions
1) Abstract: "primary" refers to tumours or tissues?

Answer: Indeed, the referee is absolutely right as this point is misleading. “Primary” relates to tumours and not to tissue. This is now changed in the revised manuscript (See abstract page 2).

2) Discussion: any further step necessary (i.e. analysis of phosphorylated CK8)?

Answer: This is an excellent point raised by the referee, which have now implemented in the discussion section (see top of page 11).
Referee: Karel Smetana

“...However, I recommend to more stress the pro-invasive phenotype of cells exhibiting K8 in discussion. K8 expression can be induced by cancer-associated fibroblasts in normal non-malignant keratinocytes. The influence of tumor microenvironment should be also discussed…”

Answer: This is an excellent point, which we have indeed over-seen in the former version of the manuscript. We have now included a section dealing with the influence of the tumour microenvironment in the regulation of K8 within the discussion. Also, we have stressed the fact that disseminated tumour cells highly expressed K8 along with the influence it might have on invasion and tissue remodelling (see page 11 for both points).
Referee: Robert G Oshima

"...While interpretation of the state of positive reactive cells is based on pattern recognition, it would be useful for the authors to summarize the critical characteristics used to make the diagnosis..."

Answer: This is again an excellent point, which was under-represented in the first version of our manuscript. Diagnosis was performed by two expert pathologists during routine appraisal of clinical and according to the UICC TNM classification of 2003 [1]. The characteristics are now described in the methods section, including adequate references (see page 5 of revised manuscript).


"...Second this study differs significantly from the study discussed in the text using disseminated disease of a mouse transgenic Neu model..."

Answer: Indeed, adenomatous epithelia are K8 positive even under normal conditions and disseminated tumour cells derived thereof simply retain their expression pattern to some degree. For the case that we describe, i.e. squamous cell carcinomas, this is different as we deal with a de novo expression. We thank the referee for this point of criticism and. We have changed the discussion section accordingly, withdrew the reference alluded to and rather mentioned de novo expression of K8 in our case.

"...The authors should reconsider the use of the CK8 abbreviation. Prevailing consensus nomenclature favor K8..."

Answer: we have exchanged CK8 for K8 throughout the manuscript in order to stick to the accepted nomenclature.