Author's response to reviews

Title: HIF1-alpha overexpression indicates a good prognosis in early stage squamous cell carcinomas of the oral floor

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Version: 3 Date: 3 June 2005

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Comments to revision:

Thank you very much for good suggestions which we included in the revised paper.

Reviewer 1:

The literature gives no uniform recommendation for a cut off point of HIF-1α. The used cut off points of HIF-1α expression varies in the literature. The Oxford Group (Beasly and Harris et al. 2002) used a cut off value for HIF-1α ≥1% in oral cancer. The Group around Semenza used cut off values for HIF-1α between 1% and 5% (Bos R, Semenza GL, van Diest PJ et al. in Cancer. 2003: HIF-1α ≥5%; Bos R, Semenza GL, van Diest PJ et al. 2001 in J Natl Cancer Inst.: HIF-1α ≥1%)

The cut off points which are in your investigations were determined by the value of HIF-1α expression of the investigated tumour samples. Other authors used a cut off point of 10 %.

With regard to the variety of the used levels, we chose three different cut off values for HIF-1α expression ≥1%, ≥5% and ≥10% , we included the description of the three levels in the chapter results. The 5% threshold of HIF-1α expression discriminated in our investigation two different populations with significant statistical differences in survival prognosis. However, in the present literature it is unknown if there exists only one cut off point for HIF-1α expression level to determine HIF-1α as a prognosticator in different treatment strategies as radiotherapy and surgical treatment, especially in view of rarely available studies with a surgical treated tumour patient group and uniform located squamous cell carcinoma. In this view we have chosen 5% as the cut off value for the main statistical analysis in our study. The respective parts have been added to the manuscript in the chapter results and discussion.

Comment to post operative condition: During the operation procedure, immediately after tumour resection, the specimens were fixed in buffered formalin (4%) overnight and immediately embedded in low melting paraffin.

Comment to cytoplasmatic staining: Obviously, we tried to include the cytoplasmatic staining of HIF-1α in our analysis but the amount of cytoplasmatic positive tumours was too small for being used for statistical analysis. The small amount of strong cytoplasmatic positive tumours specimen was seen in samples with a nuclear staining over 5% therefore we have no influence on our statistical results by including the cytoplasmatic staining. With regard to the ineffective influence of the cytoplasmatic staining level we concentrated our analysis on the nuclear expression (according to Beasley et al. 2002, Bos et al. 2001). The Oxford group (Beasly and Harris et al. 2002 Cancer Res) published a similar investigation in a surgical treated tumour patient group, they also measured only the nuclear staining and found an increased survival rate of HIF-1α positive cancer in their surgical treated patient group in concordance to our results. We included the amount of cytoplasmatic positive tumours in the chapter results.
Reviewer 2:
We are aware that the use of tissue microarrays might bias immunohistochemical results, especially in SCC’s. However, it has to be stressed that the two punches representing one tumour were taken from the centre and the periphery (tumour stromal interface) as previously described by our group for other tumour entities (Brandt et al. Cancer Res 2002). We have not seen major, statistically relevant differences between the two respective punches. These results are in line with results published by Beasley et al. (Cancer Res 2002). Using full sections, this group was able to demonstrate a rather homogeneous distribution of HIF-1 positive cells in SCC’s. In detail, no major difference could be found between perinecrotic regions and areas at the tumour-stromal interface. We are therefore strongly convinced that our results are not biased by the use of two tumour punches representing the whole tumour. The respective parts have been added to the manuscript in the chapter Method/Immunohistochemistry and the discussion.