Reviewer's report

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

Version: 1 Date: 20 April 2005

Reviewer: Alexandra A Giatromanolaki

The study by Fillies et al, comprises an adequate material and deserves publication. However, the paradoxical results reported should be attributed to a fundamental mistake related to the interpretation of HIF1a immunostaining. The authors chose to consider HIF1a+ cases the ones with nuclear reactivity in >5% of cancer cells. This is wrong, or at least fails to properly reveal the real HIF status.

HIF1a is a protein clearly synthesized in the cytoplasm and strong cytoplasmic expression is definitely a tumor-specific feature. Nuclear presence of HIF1a is certainly a marker of HIF/DNA binding but, HIF is short living in the nucleus and such a reactivity may well disappear depending on post-operative conditions till specimen inclusion in the paraffin block. The two pioneer studies reporting the production of anti-HIF Abs and immunostaining results, reported and graded separately the strong cytoplasmic staining as an indicator of HIF specific reactivity (Semenza group and Oxford group). In a large number of clinicopathological studies we performed, nuclear HIF staining failed to show any correlation with prognosis and other parameters, if separated from cytoplasmic staining. Extensive strong cytoplasmic staining is definitely a marker of HIF up-regulation and should be included as a criterion for HIF positivity. All statistics change dramatically. Inclusion of strong cytoplasmic staining in the HIF(-) group loads this group with HIF(+) cases. Another point is that 5% reactivity is very low as a cut-off point, and according to our experience and also the experience from other groups, the 10% should be used as a cut-off point to define high HIF nuclear reactivity.

Even if authors wish to focus on nuclear HIF, they are strongly recommended to provide further analysis according to a mixed nuclear/cytoplasmic staining scale, like the one reported by Giatromanolaki et al. Briefly, HIF + cases are the ones with : a. nuclear HIF reactivity in > 10% of cancer cells and/or b. cases with extensive strong cytoplasmic reactivity (in >50% of cells). If the authors will not perform such a re-analysis, we will never know if a large HIF+ group (the one with strong cytoplasmic staining) was in fact included in the HIF- group, leading to erroneous prognostic correlations.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests: no