Reviewer's report

Title: Carbonic anhydrase IX in oligodendroglial brain tumors

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Reviewer: Matthias Preusser

Reviewer's report:

General

The authors present a retrospective study on CAIX expression and its correlation with expression of antioxidative enzymes, tumor cell proliferation and patient outcome in oligodendroglial brain tumors. They report a independent association with patient outcome and an inverse correlation with cell proliferation. The patient cohort is relatively large, considering that oligodendroglial neoplasms are rare. The manuscript is clearly written, concise and easy to follow. However, there are several problems that need to be addressed.

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

The authors describe that CAIX and AOE immunostaining was divided into four categories: no, weak, moderate, strong immunostaining. Do these categories refer to staining intensity or staining extent (in my opinion, extent of staining would be more meaningful)? In this context, Figure 1 depicting CAIX immunoreactivity is of concern. I feel that most observers experienced in evaluation of immunostaining would not regard B and C as unequivocally positive.

The authors should more precisely define the evaluation categories (e.g. strong immunostaining = more than 50% of tumour tissue immunostained, etc.). Assessment of immunostainings should be performed by at least two independent observers to demonstrate reproducibility of assessment. A statistical measure, for example kappa values, should be provided to demonstrate observer agreement.

The results section should contain a passage describing CAIX immunolabelling. Is the signal on the cell surface or does it stain the cytoplasm? Which areas in the tumour are labelled? Our group has used the same anti-CAIX antibody in a series of 1p-aberrant oligodendroglial neoplasms (Preusser et al. DEC1 expression in 1p-aberrant oligodendroglial neoplasms. Histol Histopathol. 2005 Oct;20(4):1173-7). In this study perinecrotic accentuation of CAIX expression was demonstrated. A reprint of this article is available upon request at matthias.preusser@meduniwien.ac.at, if the authors wish to cite it.

Does CAIX-positivity correlate with presence of necrosis or presence of microvascular proliferations?

The authors state that the proliferative activity was reported as percentage of immunopositive nuclei on anti-Ki67 immunostained slides. Please provide more details on the method of assessment- was the percentage of positive nuclei determined by counting (if yes, how many nuclei were counted)? Which area was assessed- "hot spot" showing the highest density of immunolabelled nuclei?

Regarding the proliferation index, the authors report a median of 2.9% for CAIX+ and a median of 5.8% for CAIX- tumours. In our experience, there is substantial inter- and intraobserver variability in assessment of MIB1 proliferation index. It should be demonstrated whether the MIB1 proliferation index still correlates with CAIX expression when assessed by a different observer, who is blinded to all previous results. If the correlation of low MIB with high CAIX expression holds true, the authors should discuss possible explanations for this inverse association.

The assessment of TUNEL stained sections should be described in more detail.

CAIX expression was significantly higher in MnSOD-positive tumours, which is interesting because, as the authors write in the discussion, CAIX is induced by hypoxia and MnSOD by hyperoxia. How was the spatial relationship between CAIX-positive and MnSOD-positive areas? This question should be answered by using immunostaining on adjacent sections or double immunolabelling.

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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)

The authors state that histological component (pure oligodendroglioma vs. mixed oligoastrocytome) showed independent prognostic significance. Please specify which of these two diagnoses had more favourable outcomes.

Reference 34 is cited as a source on information on antibody M75. As far as can be told from the title and the medline abstract, this reference does not seem to relate to CAIX. Please check whether this is the correct reference.

Discretionary Revisions (which the author can choose to ignore)

More information on the treatment of the patients would be of interest.

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 limited interest

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.