Reviewer’s report

Title: Comparative analysis of histologically classified oligodendrogliomas reveals characteristic molecular differences between subgroups

Version: 3 Date: 03 Mar 2018

Reviewer: Aaron Diaz

Reviewer's report:

The authors state: "We understand that there are currently different opinions about how to weight histology and molecular markers for glioma classification." However, this statement is false. Genotype always trumps histological phenotype. Please see the following: "The diagnostic use of both histology and molecular genetic features also raises the possibility of discordant results, e.g., a diffuse glioma that histologically appears astrocytic but proves to have IDH mutation and 1p/19q codeletion, or a tumor that resembles oligodendroglioma by light microscopy but has IDH, ATRX and TP53 mutations in the setting of intact 1p and 19q. Notably, in each of these situations, the genotype trumps the histological phenotype, necessitating a diagnosis of oligodendroglioma, IDH-mutant and 1p/19q-codeleted in the first instance and diffuse astrocytoma, IDH-mutant in the second." Louis et al. Acta Neuropathologica. 2016 The above review summarizes WHO 2016. It was authored by participants of the Consensus and Editorial Conference, whose input formed the basis of the 2016 WHO edition. Point-by-point:

1. The authors say: "We respectfully disagree. In our opinion, the discussion about how to weight histological and molecular markers for glioma classification is very important.

Genotype always trumps histological phenotype. Please see the following: "The diagnostic use of both histology and molecular genetic features also raises the possibility of discordant results, e.g., a diffuse glioma that histologically appears astrocytic but proves to have IDH mutation and 1p/19q codeletion, or a tumor that resembles oligodendroglioma by light microscopy but has IDH, ATRX and TP53 mutations in the setting of intact 1p and 19q. Notably, in each of these situations, the genotype trumps the histological phenotype, necessitating a diagnosis of oligodendroglioma, IDH-mutant and 1p/19q-codeleted in the first instance and diffuse astrocytoma, IDH-mutant in the second." Louis et al. Acta Neuropathologica. 2016 The above review summarizes WHO 2016. It was authored by participants of the Consensus and Editorial Conference, whose input formed the basis of the 2016 WHO edition. Point-by-point:

1. The authors say: "We respectfully disagree. In our opinion, the discussion about how to weight histological and molecular markers for glioma classification is very important." This was relevant a 5-10 years ago, before the defining mutations of astros and oligos were elucidated (e.g. https://www.ncbi.nlm.nih.gov/pubmed/26061753 ). With the recent work of Venteicher 2016, we now also know that oligos and astros are phenotypically the same at the level of transcription, other than differences due to genetics. The position of the WHO is that genotype trumps the histological phenotype, which settles the discussion of how to weigh the two. 2. The authors claim they cannot compare oligos to astros because they would need a pathologist to assess the morphology. However, this is also false, since genotype trumps histological phenotype and genotype data is freely available. 3. The authors quote the mention of histology in Venteicher 2016, however, they do not report the primary finding of that paper. Venteicher 2016 found that the only phenotypic differences between oligos and astros were due to differences in genetics (all differentially expressed genes were expressed from 1p, 19q, or were targets of p53). Thus, the primary result of Venteicher 2016 confirms the WHO 2016 interpretation that the only differences in phenotype can be explained by genetics, and therefore the principle that genotype should trump the histological phenotype in defining the molecular subtypes of IDH-mutant glioma. 4. Regarding the significance of the differential expression test: SOX6 and SOX8 are well studied regulators of oligodendrocyte maturation, e.g.: http://www.cell.com/developmental-cell/fulltext/S1534-5807(06)00357-1https://www.ncbi.nlm.nih.gov/pubmed/15893981https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2767240/ It is therefore not surprising to see SoxC/D enriched in oligodendrogliomas. Since no follow-up functional assays were performed, no mechanism for any gene was learned. The significance of this finding is minimal. Moreover, the current comparison of astros to oligos is unbalanced in terms of sample size. This is a technical caveat that I raised in my initial review, again in my second review. It is still
unaddressed even though there are numerous techniques to adjust for unbalanced sample sizes. The authors have not provided a rationale for not addressing this critical issue. Lastly, no conclusions have been drawn from this result. In summary, the authors have computed CNVs in oligos and astros and showed that they cluster by the 1p/19q co-deletion. This result has been shown previously and is partially the basis for current WHO glioma classification. Honestly, I have wanted to be supportive of this manuscript from the beginning, and of the author's research agenda of studying copy-number alterations in glioma. However, the authors have not addressed any of my concerns, and have only made cosmetic changes to the manuscript. Moreover, there is a fundamental misunderstanding of glioma subtypes that is expressed in the current manuscript that would serve only to add confusion to the literature.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
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No

Are the conclusions drawn adequately supported by the data shown?
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