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IDH mutant astrocytoma: biomarkers for prognostic stratification and the next frontiers

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The discovery of *IDH* mutations in a subset of glioblastomas 10 years ago [1] has fundamentally changed the approach of brain tumour diagnostics. Soon after this discovery, it emerged that low- and high-grade oligodendrogliomas, diffuse and anaplastic astrocytomas, a proportion of glioblastomas and the now defunct oligoastrocytomas carry mutations in the *IDH1* or *IDH2* genes [2]. Subsequently, the IDH-mutant tumours were stratified with additional biomarkers, which has led to the definition of the WHO (2016) classes IDH-mutant, 1p/19q codeleted (and *TERT* promoter mutant) oligodendroglioma and IDH-mutant astrocytoma, with its characteristic loss of ATRX protein expression, assessed by immunostaining [3]. However, there remain challenges in the group of IDH-mutant astrocytic tumours: their clinical behaviour varies widely and does not always overlap with the histological criteria of malignancy [4]. In particular, small foci of microvascular proliferation in otherwise low-grade appearing IDH-mutant astrocytomas or necrosis in recurrent astrocytomas treated with radiotherapy, can present diagnostic dilemmas with implications for clinical management. Recently, homozygous deletions of *CDKN2A/B* were identified as a biomarker that further stratifies IDH-mutant astrocytic tumours: those with a *CDKN2A/B* homozygous deletion do much worse clinically than non-deleted tumours, even if there are no apparent histological high-grade features [5].

In their study in this issue, Korshunov *et al.* [doi To editorial office: please insert DOI or another suitable reference] analysed a group of 97 IDH-mutant glioblastomas (GBM-IDH) with complementary molecular methods. Copy number variations and epigenetic profiles of these tumours were analysed with Illumina methylation arrays and compared to a dataset of IDH-wildtype GBM, IDH-mutant lower grade astrocytomas and IDH-mutant oligodendrogliomas. In addition to targeted sequencing of *IDH1, IDH2* and the *TERT* promoter and immunohistochemical staining for ATRX, all tumours underwent next generation sequencing (NGS) with a panel of 130 cancer-associated genes.

They found that the majority of the “de novo” GBM-IDH and the “evolved" GBM-IDH form a group that is distinct from lower grade IDH-mutant gliomas. Of the 97 GBM-IDH, nearly a third had a pre-existing histologically confirmed lower grade tumour, obtained through stereotactic biopsy. These had been treated with radiotherapy only and had progressed within 3-5 years to GBM-IDH (i.e. evolved to GBM-IDH). The remaining 68 patients presented with a “de novo” GBM-IDH, characterised by a short clinical history. Intriguingly, this study shows that there are no differences in

the genetic or epigenetic signature within the group of GBM-IDH, regardless of whether they present "de novo" or evolve from lower grade astrocytomas (Fig 1).

To inform oncologists of the most effective treatment, it is essential to provide a pathological diagnosis that contains useful prognostic information. Methylation arrays have been instrumental in stratifying brain tumours in prognostically more relevant entities [6, 7] (Fig 1). By t-Distributed Stochastic Neighbour Embedding (t-SNE) dimensionality reduction, the majority of GBM-IDH tumours in this study were allocated to IDH-mutant glioblastoma cluster. However, 11 tumours corresponding histologically to GBM were allocated to the lower grade IDH-mutant astrocytic glioma cluster (Fig 1). t-SNE clustering was also used previously in establishing methylation classes with the brain tumour methylation classifier [7]. t-SNE depends (among other parameters) on the number of cases and the comparators, for example different entities. Whilst for the majority of samples, the results of t-SNE clustering and subsequent allocation to high-grade or low-grade astrocytoma aligns with the DKFZ methylation classifier results, there can be discrepancies for a small number of cases. By comparing differentially methylated sites between tumours corresponding to low-grade and high-grade IDH-mutant astrocytoma methylation clusters, the authors identified that differences were found in the pathways involving receptor tyrosine signalling, "neuronal system" and extracellular matrix organisation. The diagnostic or scientific utility of this finding is not yet clear.

No difference in overall survival was observed between tumours arising "*de novo*" or evolving from lower grade tumours, or between tumours corresponding to different methylation clusters, but this may be due to small sample size. However, the role of *CDKN2A/B* homozygous deletion in this study has been further corroborated as a prognostic biomarker conferring shorter survival in GBM-IDH [5]. Only one of the GBM-IDH, corresponding to lower grade astrocytoma according to t-SNE, harboured a *CDKN2A* homozygous deletion, and undoubtedly the deletion of *CDKN2A/B* will become a defining biomarker in upcoming consensus discussions (Fig 1).

This work adds to our understanding of genetic and epigenetic alterations in IDH-mutant astrocytomas. More work needs now to be done to characterise rare molecular constellations which still present diagnostic ambiguities, and which may have clinical prognostic implications. Notably, the relevance (if any) of *TERT* promoter mutations in a small proportion of IDH-mutant astrocytomas is an important finding which must be recognised during diagnostic workup of diffusely infiltrative gliomas.

The loss of nuclear ATRX protein expression is an important diagnostic biomarker for IDH-mutant astrocytic tumours [8, 9]. It needs to be established in larger cohorts how the *ATRX* mutations identified in the NGS panel match with the loss of the protein expression, and how many IDH-mutant high grade astrocytomas with retained nuclear ATRX expression (sometimes creating a diagnostic ambiguity) would benefit from NGS panels in identifying functionally relevant genomic *ATRX* mutations. Also, the possibility of co-occurrence (or indeed as previously suggested mutual exclusivity) of *ATRX* mutations and *TERT* promoter mutations and their relation to tumour biology needs clarification in well-characterised cohorts like this. This publication shows the great utility of methylation arrays in particular in combination with datasets generated by NGS panels, and at the same time highlights the limitations of genomic studies in isolation without the information from epigenetic profiling.

Building upon this comprehensive dataset of IDH-mutant glioblastomas, the next frontiers will be the validation of these findings in much larger, multicentre cohorts and the characterisation of the diagnostic and prognostic relevance of unusual, rare combinations of mutations, copy number

variations and epigenetic alterations. This knowledge is also an essential basis for research into imaging biomarkers and in stratifying patients for clinical trials and tailored treatments.

Figure legend

Figure 1: Proposed simplified algorithm of risk stratification and prognostication of IDH-mutant glioma, based on studies by Shirahata *et al.* [5] (light blue shade), with layered data from Korshunov *et al.* (this issue, light orange shade). *Upper rows:* unsupervised hierarchical clustering of DNA methylation profiles groups together nearly all (96/97) histologically diagnosed IDH-mutant glioblastomas (GBM-IDH) (dark red) and separates them from lower grade IDH-mutant astrocytomas (dark green). *De novo* and evolving IDH-mutant glioblastomas cluster in the same group (top row, dark red) and most of them (84/97) also correspond to the methylation cluster of high grade IDH-mutant glioblastoma according to t-SNE analysis (light red, second row). Notably, a small proportion (11/97) of histologically diagnosed GBM-IDH (dark red, top row) overlaps with the methylation cluster of lower grade IDH-mutant astrocytomas (second row, light green) and one case clusters together with oligodendroglial tumours (grey, second row) despite absence of 1p/19q codeletion. GBM-IDH in this study comprises tumours with and without *CDKN2A/B* homozygous deletion (data not shown). *Middle rows* ("biomarkers"): By definition, all tumours are IDH-mutant (orange) and most show loss of nuclear ATRX expression (purple). Occasionally ATRX expression is retained, and in these instances 1p/19q testing is recommended to confirm 1p/19q non-codeletion (light purple). As proposed by Shirahata *et al*. [5], IDH-mutant astrocytic gliomas corresponding to WHO grade II to WHO grade IV ("histology"), are further stratified for prognostication by *CDKN2A/B*, copy number variation (CNV) and necrosis. Tumours with intact *CDKN2A/B*, no CNV and no necrosis have more favourable prognosis and correspond to "low malignancy astrocytomas, (*bottom rows*, integrated diagnosis and prognosis). Tumours with intact *CDKN2A/B*, high CNV and/or necrosis correspond to intermediate malignancy astrocytoma. Tumours with homozygous deletion of *CDKN2A/B*, regardless of CNV or presence of necrosis, correspond to high malignancy astrocytomas (astrocytoma, grade 4 in [5]). The malignancy grades in this classification overlap to some extent, but do not fully match the current histological WHO (2016) grades, which do not account for the prognostically relevant biomarkers as proposed by [5].

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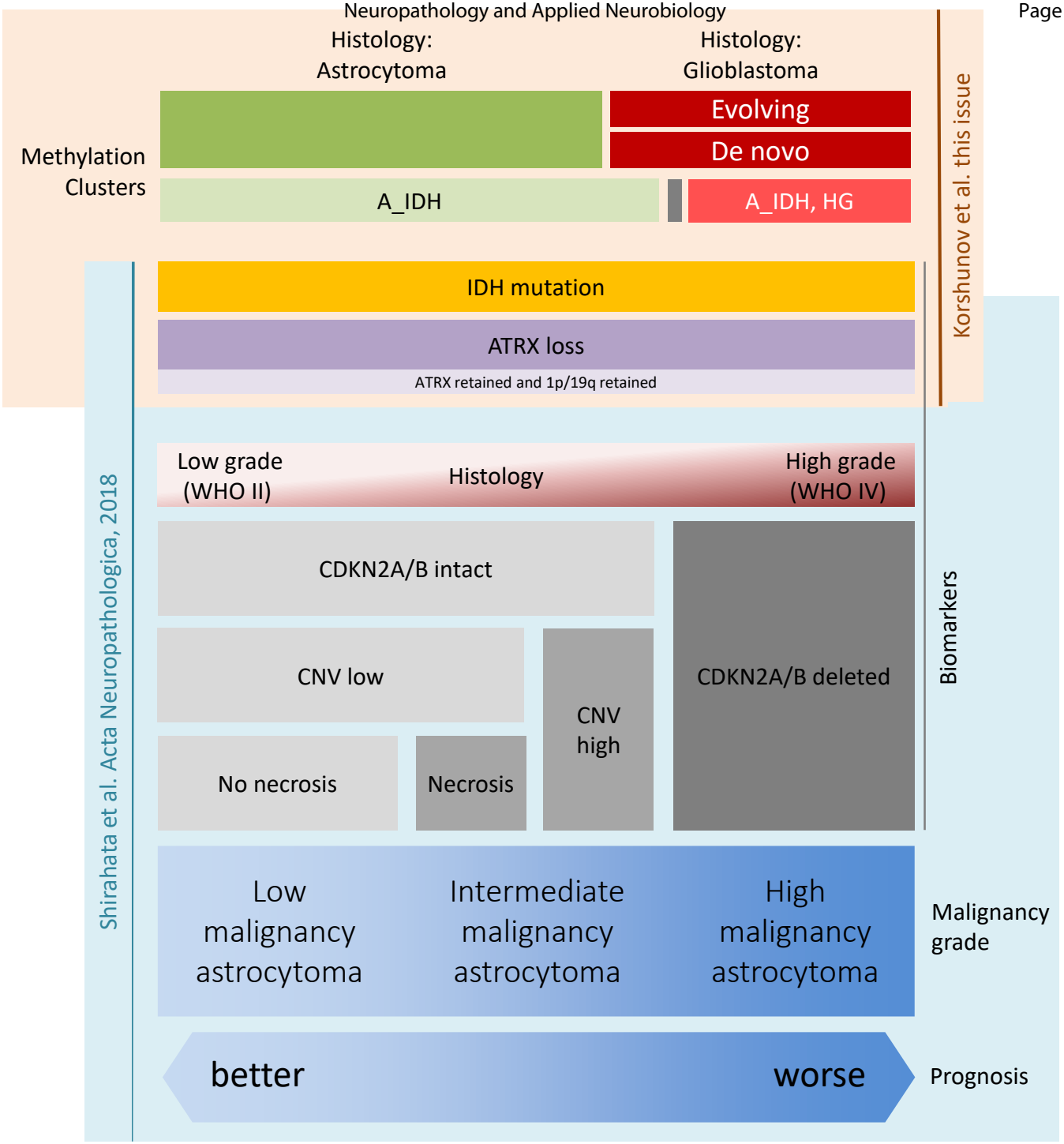
Histology: Astrocytoma
Histology: Glioblastoma
Evolving
De novo
Methylation Clusters
A_IDH
A_IDH, HG
Korshunov et al. this issue
IDH mutation
ATRX loss
ATRX retained and 1p/19q retained
Low grade (WHO II)
Histology
High grade (WHO IV)
CDKN2A/B intact
CNV low
No necrosis
Necrosis
CNV high
CDKN2A/B deleted
Biomarkers
Shirahata et al. Acta Neuropathologica, 2018
Low malignancy astrocytoma
Intermediate malignancy astrocytoma
High malignancy astrocytoma
Malignancy grade
better
worse
Prognosis