A review of molecular alterations with clinical impact in adult and paediatric gliomas

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Abstract

The practice of neuropathology underwent major changes due to the discovery of molecular alterations with diagnostic, prognostic, and therapeutic implications. Many of these alterations are incorporated in the updated 2016 edition of the World Health Organization (WHO) Classification of Tumours of the Central Nervous System (CNS), which represents a shift from the principle that neuropathology diagnosis is based entirely on microscopy. Molecularly defined entities were introduced, and a diagnosis that integrates the histology and molecular results is strongly recommended. This complex approach is timely because the neuro-oncologists seek specific diagnoses that can lead to more precise treatment. In the current review, we present the most common molecular alterations with known clinical implications in gliomas, and provide a practical guide to an integrated diagnosis.

Keywords *BRAF V600E*; *CDKN2A*; *H3 K27M*; *IDH*; integrative diagnosis; *MYB*; paediatric brain tumours; *RELA*

Introduction

In the past decade, the practice of neuropathology has shifted from the traditional approach to brain tumour classification based primarily on microscopic features, to becoming driven by the tremendous amount of recent genetic/genomic and epigenetic discoveries. These discoveries have led to a better understanding of tumour biology, prognosis, and therapeutic options. The arrival of the updated 2016 edition of the WHO Classification of the Tumours of the CNS is timely because, similar to the WHO Classification of Tumours of Hematopoietic and Lymphoid tissues, it provides an integration of the histologic and molecular findings, while maintaining a standardized approach to diagnosis.[1,2] The classification of diffuse gliomas and embryonal tumours underwent the most significant changes, with the former being classified based on their IDH and 1p/19q status (Table 1), and the latter comprising medulloblastomas with well-recognized molecular classes (Wnt, Sonic Hedgehog (SHH), class 3 and 4), addressed in another chapter of this edition. The diagnosis of oligodendroglioma is now defined by the co-existence of IDH mutation and 1p/19q codeletion, which vastly diminishes the diagnosis of oligoastrocytoma. The diagnosis of oligoastrocytoma is now reserved only for tumours that have a demonstrable double phenotype and molecular profile, resulting in a more objective approach to diagnosis, with less inter-observer variability. In addition to major changes in the overall classification of the CNS tumours, new entities with unique biology and prognostic implications have been introduced, some defined by signature molecular alterations. For example, it became necessary to know the status of *H3 K27M* mutation in a midline diffuse glioma and the status of *C11orf95-RELA*-fusion in hemispheric ependymomas, since these alterations are associated with poor prognosis.

Low-grade gliomas and low-grade glioneuronal tumours are an important category of neoplasms in children and young adults and occasionally they are referred to collectively as low-grade neuroepithelial neoplasms. Although their prognosis is generally good, sometimes these tumours have an infiltrative pattern of growth and/or their location precludes a complete resection. For some of them, the driver genetic alterations have been known for a long time: pilocytic astrocytoma and pilomyxoid astrocytoma have well known described alterations in MAPK pathway (most commonly *BRAF-KIAA1459* fusion, but also *NF1* mutations, *BRAF V600E* mutation, *BRAF* intragenic deletions, and *BRAF* fusions with other partners). Over the past years our understanding of low-grade neuroepithelial tumours has expanded, and we now know that more than 80% of pleomorphic xanthoatrocytomas and 30–50% of gangliogliomas are associated with *BRAF V600E* mutations.[3] It is also known that the molecular alterations present in adult diffuse gliomas (IDH mutations, 1p/19q codeletion, ATRX mutations) are rare before puberty, and there is a large number of paediatric gliomas for which we have just begun to understand the molecular drivers and their prognostic implications.

We are at a time in the practice of neuropathology where it is important to incorporate molecular results in our reports in order to offer a precise treatment for our patients. We present a review of practical aspects related to tissue triaging, and interpretation of essential molecular tests used in the diagnosis of glioma.

Tissue triaging in neuropathology specimens

The neuropathologist's first encounter with a brain tumour specimen is often times at the time of the frozen section. In addition to rendering a preliminary diagnosis, this is the time when plans of tissue triaging are made, so that the necessary immunohistochemical and molecular tests can be performed. Although practice and availability of tests vary from institution to institution, most of the methods necessary for the molecular characterization of CNS tumours can be performed on formalin-fixed paraffin-embedded tissue. Most brain tumours have single nucleotide variations and copy number changes that can be determined through surrogate immunohistochemical stains, fluorescence *in situ* hybridization (FISH), single variants tests (pyrosequencing), and DNA-based sequencing platforms. The rare chromosomal rearrangements of interest in neuropathology can be determined through FISH or sequencing, therefore obviating the need for karyotype analysis.

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2016 WHO Classification of diffuse astrocytic and oligodendroglial tumours

Diffuse astrocytoma, *IDH-mutant*, WHO grade II or III
Diffuse astrocytoma, *IDH-wild type*, WHO grade II or III
Diffuse astrocytoma, NOS, WHO grade II or III
Oligodendroglioma, *IDH-mutant*, 1p/19q-codeleted, WHO grade II or III
Oligodendroglioma, NOS, WHO grade II or III
Oligoastrocytoma, NOS, WHO grade II or III
Glioblastoma, *IDH-mutant*, WHO grade IV
Glioblastoma, *IDH-wild type*, WHO grade IV
Giant cell glioblastoma
Gliosarcoma
Epithelioid glioblastoma
Glioblastoma, NOS, WHO grade IV
Diffuse midline glioma, *H3 K27M-mutant*, WHO grade IV

Table 1

The only situation when karyotype might be useful is if during the frozen section the neuropathologist is confronted with the possibility of a mesenchymal, soft tissue-type solid tumour. Tissue requirements for molecular testing are based on the method used: immunohistochemical stains are widely used and the easiest to implement; this method requires a single 4 microns unstained section. Multiple 4 microns unstained sections (usually up to ten) or tissue scrolls of a representative tumour block with at least 50% tumour nuclei are needed for most other molecular methods. Therefore, prioritizing tissue for tests that have the highest likelihood to lead to a specific neuropathologic diagnosis and treatment plan is important, especially in small biopsy specimens.

Additionally, a large number of patients with CNS neoplastic diseases consent to research protocols and clinical trials either before or after surgery. Therefore, freezing a small fragment of lesional tissue, if available, is often required.

Must-know alterations in paediatric and adult gliomas

Isocitrate dehydrogenase (IDH) mutations

IDH1 and *IDH2* encode a particular metabolic enzyme in the Krebs cycle, and somatic mutations are encountered in more than 70% of diffuse astrocytomas and oligodendrogliomas WHO grade II and III, and in secondary glioblastomas. These mutations are rare in other brain tumours, making it a useful marker when the differential diagnosis includes pilocytic astrocytoma, pleomorphic xanthoastrocytoma, primary glioblastoma, reactive astrogliosis, and other CNS tumours with oligodendroglial morphology. The majority of *IDH* mutations involve the *IDH1* gene, and the most common mutation is an arginine 132 to histidine substitution (R132H). However, absence of *IDH1* (R132H) mutation does not preclude the presence of other *IDH* mutations, especially in young patients, who can have mutations in *IDH2* gene. In general, patients with *IDH* − mutant diffuse gliomas are younger and have a better prognosis than those with *IDH*-wild type diffuse gliomas. Recent studies demonstrate very little differences in the prognosis of grade II and grade III *IDH*-mutant diffuse astrocytomas, which poses substantial challenges in the practice of astrocytoma grading and in the management of the patients.[4] Since the presence of *IDH* mutations is correlated with a better prognosis and better response to treatment, it is strongly recommended that diffuse gliomas be investigated for mutation status. The mutation-specific immunohistochemical antibody IDH1 (R132H) (clone H09) is a reliable method of detecting the mutation (Figure 1a). An inconclusive result, or absence of *IDH1* mutation by immunohistochemistry in a diffuse glioma, should prompt follow-up with sequencing for both *IDH1* and *2*, especially in a young adult. Although mutations in *IDH* are frequent in diffuse gliomas in the adult population, they are rare before puberty.[5]

1p/19q codeletion

The codeletion of 1p/19q due to an unbalanced translocation t (1;19) (q10:p10), along with *IDH* mutation, is a requirement for the diagnosis of oligodendroglioma in the updated 2016 WHO Classification of the CNS Tumours (Table 1). 1p/19q codeletion and *ATRX* mutations are mutually exclusive; when used together in the diagnostic workup of diffuse gliomas, these alterations can provide the distinction between oligodendrogliomas, which have 1p/19q codeletion and *IDH* mutations, and diffuse astrocytomas, which have both *IDH* and *ATRX* mutations, but do not exhibit 1p/19q codeletion. Occasionally, the morphology does not correlate with the molecular test results. For example, some tumours demonstrate an oligodendroglial morphology, but an astrocytic molecular profile, with *ATRX* mutation and absence of 1p/19q codeletion. For such situations, the WHO recommendation is that the molecular diagnosis trumps the histology. Oligodendrogliomas with 1p/19q codeletion have been associated with a favourable prognosis, and, depending on each neuro-oncology practice, this finding might lead to modifications in the therapeutic protocol. Tumours with 1p/19q codeletion are rare in paediatric patients. If present, this alteration does not necessarily carry the same prognostic implications as in the adult population.

Probably the most available method of detection of 1p/19q codeletion is fluorescence *in situ* hybridization (FISH), which uses dual-colour commercial probes for the subtelomeric regions of 1p and 19q. Array comparative genomic hybridization (CGH) is a DNA-based genome-wide test available clinically in some laboratories, and appears to be a more specific method than fluorescence *in situ* hybridization because it confirms the loss of whole chromosome arm, which cannot be demonstrated by FISH. In addition, array CGH provides information about other structural chromosomal abnormalities, as well as copy number aberrations. Both methods described use formalin-fixed paraffin-embedded tissue (FFPE). Other methods include various DNA-based sequencing platforms with breaKmer, which also provides information about other single nucleotide variants found in diffuse astrocytomas and oligodendrogliomas (e.g. *ATRX*, *TP53*, *CIC*, *FUBP1*, etc), as well as potential information about signalling cellular pathways that can be triggered by therapy (e.g. *PIK3CA/Akt, MDM2, Rb, PDGFR* alterations, etc).

Alpha thalassemia/mental retardation X-linked(ATRX) and telomerase reverse transcriptase (TERT) promoter mutations

Telomeres are repetitive nucleotide sequences at each chromatid end, and normally they shorten with each cell division. In

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