Molecular background of oligodendroglioma: 1p/19q, IDH, TERT, CIC and FUBP1

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Practice points

- All 1p/19q codeleted oligodendrogliomas are IDH1/2 mutant.
- Genomic alterations in the TERT promoter sequence and the *CIC* and *FUBP1* genes often accompany 1p/19q codeletion and IDH1/2 mutation, reinforcing the molecular evidence base supporting a discrete diagnosis.
- 1p/19q codeleted oligodendrogliomas are responsive to adjuvant treatment with radiation and procarbazine, CCNU (lomustine) and vincristine chemotherapy.

Oligodendroglioma is the quintessential molecularly-defined brain tumor. The characteristic whole-arm loss of the long arm of chromosome 1 and the short arm of chromosome 19 (1p/19q-codeletion) within the genome of these tumors facilitated the reproducible molecular identification of this subcategory of gliomas. More recently, recurrent molecular genetic alterations have been identified to occur concurrently with 1p/19q-codeletion, and definitively identify these tumors, including mutations in *IDH1/2*, *CIC*, *FUBP1*, and the *TERT* promoter, as well as the absence of *ATRX* and *TP53* alterations. These findings provide a foundation for the consistent diagnosis of this tumor type, upon which a generation of clinical investigators have assembled a strong evidence base for the effective treatment of this disease with radiation and chemotherapy.

Oligodendrogliomas are the second most common intraparenchymal brain tumor in adults [1]. Classified under WHO neuropathologic criteria, oligodendrogliomas can be either low-grade (grade II) or anaplastic (grade III) [2]. Long-recognized as a distinct histological entity within the family of diffuse gliomas [3,4], oligodendrogliomas were established as the prototypic 'molecularly-defined' brain tumor by a series of discoveries at the end of the 20th century. These clinical-translational studies linked the recognition of a marked chemotherapeutic responsiveness and long survival in histologically typical anaplastic oligodendrogliomas [5–7] with the characteristic genomic alteration of whole-arm chromosomal loss of 1p and 19q in these gliomas [8,9].

With the advent of large-scale genome sequencing technology, molecular genetic alterations in *IDH1/2*, *CIC*, *FUBP1* and the *TERT* promoter have now been identified in the majority of oligodendrogliomas. These alterations have afforded investigators with an increasing number of overlapping and mutually reinforcing criteria to diagnose these lesions. Similarly, genetic mutations identified as characteristic of other subgroups of diffuse gliomas, such as *ATRX* and *TP53* mutations in astrocytomas, have allowed for molecular tests that can nearly exclude the diagnosis of oligodendroglioma. In aggregate, these discoveries have led to a greater appreciation of the limits

KEYWORDS

- 1p/19q loss • *CIC* mutation • *FUBP1* mutation • *IDH* mutation • oligodendroglioma • *TERT* mutation

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of both histologic sampling [10,11] and molecular diagnostic testing [12–14]. Indeed, it seems likely that the diagnosis of oligodendroglioma will become one in which histopathology and molecular diagnosis are combined.

Herein, we review these molecular discoveries, and the resulting lessons for the diagnosis of oligodendroglioma. We underscore the emerging ‘combined consensus’ definition of the entity of oligodendroglioma, incorporating the presence (and/or absence) of specific molecular genetic alterations alongside demographic data, clinical information and histopathologic review. Not discussed in this review are possible MRI correlates of oligodendroglioma histopathology and genetics, but several groups have pointed to apparent/associations between the location of oligodendrogliomas, their appearance on MRI and the presence (absence) of 1p/19q co-deletion and mutation of *IDH1/2*.

Oligodendroglioma histology & 1p/19q status

The therapeutic implications of the tight linkage between histopathologic appearance and molecular genetics in oligodendroglioma have been firmly established in a number of studies since the initial report [9]. It is worthwhile to review this existing evidence base as established by several completed and ongoing Phase III studies.

For anaplastic oligodendroglial tumors (WHO grade III), a histopathology of ‘pure’ anaplastic oligodendroglioma (AO) – versus the ‘mixed’ histology anaplastic oligoastrocytoma (AOA) – has proven to be an important distinction. In the EORTC 26951 study, a ‘pure’ AO histology identified tumors with a better prognosis, and predicted improved outcome after receipt of the chemotherapeutic regimen of procarbazine, CCNU (lomustine) and vincristine (PCV) when administered as adjuvant treatment in addition to a standard course of radiotherapy (RT). For patients with AO [15], overall survival (OS) associated with receipt of RT and PCV was significantly prolonged (3.5 vs 2.6 years compared with RT alone, a hazard ratio [HR]: 0.75; 95% CI: 0.60–0.95). However, there are limits to the predictive capacity of histology, since in the RTOG 9402 study of AO and mixed AOA [16], the median survival (though longer overall when compared to the EORTC cohort) was not different between patients receiving PCV plus RT (4.6 years) versus RT alone (4.7 years); HR: 0.79; 95% CI: 0.60–1.04.

Indeed, the molecular diagnosis of combined 1p/19q loss has proven to be a critical piece of this puzzle, identifying a cohort of tumors in both studies that responded to the addition of PCV to RT. The RTOG 9402 study observed an associated extension of median survival to 14.7 years (from 7.3 years) in this subgroup with combined 1p/19q loss; HR: 0.59; 95% CI: 0.37–0.95; $p = 0.03$. A similar finding was reported in the EORTC study, in which the median OS was not yet reached in the 1p/19q-deleted RT/PCV group, versus 9.3 years in the RT group; HR: 0.56; 95% CI: 0.31–1.03. An early report from the ongoing EORTC trial 22033–26033 of RT versus temozolomide (TMZ) chemotherapy in low-grade gliomas (grade II) has noted a similar prognostic association of chromosome 1p deletion with prolonged progressive-free survival ($p = 0.0003$; HR: 0.59; 95% CI: 0.45–0.78) and OS ($p = 0.002$; HR: 0.49; 95% CI: 0.32–0.77).

Thus, the glioma identified as ‘oligodendroglioma’ by histopathologic information alone is better-identified as a clinically actionable entity by the additional molecular scoring of 1p/19q status. Conversely, 1p/19q testing alone is not sufficient, as 1p/19q loss is not prognostic in non-oligodendroglial lesions [17]. With the explosion of molecular genetic discoveries within these tumors (detailed below), recent authors have attempted to explicitly render this distinction between the underlying neoplastic entity and the histopathologic diagnosis, using such consensus designations as ‘molecular oligodendroglioma’ [18], or ‘ICF glioma’ [19]. Oligodendrogliomas thus have served as the prototypic example of a molecularly refined clinicopathologic entity, underscoring the need for multiple lines of evidence to identify the clinically relevant neoplasm.

Genetic mutations as subclassifiers

In the last decade, molecular genetic sequencing methods have undergone a rapid technological advancement. As a result, several recurrent mutations have been identified in diffuse gliomas, allowing for a molecular re-evaluation of histologic subclassification systems.

• IDH

Application of genome-scale sequencing techniques to malignant gliomas identified mutations in the isocitrate dehydrogenase gene *IDH1* in a subset of glioblastomas [20]. On closer inspection, these *IDH1*-mutant tumors proved to be

glioblastomas arising in younger patients from previously diagnosed lower-grade gliomas [20–23]. This discovery had immediate implications for the understanding of different WHO grades as discrete lesions, as low-grade gliomas retain the *IDH1* gene mutation and continue to have an improved prognosis, despite histologic grade progression. Thus, *IDH1* mutation occurs early within the evolutionary development of a distinct molecular etiologic class of glioma [24–26].

Approximately 90% of these alterations occur within a ‘hotspot’ of arginine to histidine heterozygous mutations in codon 132 of the *IDH1* gene (R132H), with the remainder as noncanonical substitutions in *IDH1* (e.g., R132C or R132S) or in the homologous gene *IDH2* (R172K). All tumor-associated *IDH* mutants acquire a neomorphic enzymatic activity resulting in the overproduction and near-100-fold accumulation of the R enantiomer of 2-hydroxyglutarate (also known as ‘D’ enantiomer or D-2-HG) [27]. The high concentration of 2-HG competitively inhibits a large family of 2-oxoglutarate (also called α -ketoglutarate)-dependent dioxygenases, and is thought to promote tumorigenesis by impacting chromatin structure and the epigenetic control of gene expression associated with the glioma CpG island methylator phenotype [28–30]. Pharmacologic inhibition of this mutant enzymatic activity may offer a targeted therapeutic strategy for these gliomas [31]; this is an area of active investigation.

Currently, however, IDH mutation status has already proven useful as a diagnostic tool for gliomas. A true clinical-translational breakthrough came with the introduction of a mutation-specific antibody directed against the most common IDH1 mutation (R132H, seen in >90% of mutant cases) by Dr von Deimling’s laboratory group, allowing for rapid large-scale immunohistochemical screening of formalin-fixed specimens and widespread diagnostic assessment of IDH R132H status as part of routine neuropathologic practice [15,32–36]. The antibody is now in widespread use clinically, and can now be used to distinguish reactive gliosis from a diffusely infiltrating, low cellularity, grade II glioma and to distinguish diffuse grade II gliomas from glioneuronal tumors like gangliogliomas, dysembryoblastic neuroepithelial tumor (DNET) or clear-cell ependymoma [37,38]. It is of course important to reiterate that this antibody does not detect noncanonical IDH1 mutations

such as R132C, R132S, R132G, R132L or IDH2 mutations.

Despite their distinct histologic differences, both lower-grade astrocytomas and oligodendrogliomas have high rates of *IDH* gene mutations, exceeding 80% in most series. For oligodendrogliomas, the linkage between *IDH* gene mutation and histology is particularly tight; the few oligodendrogliomas that do not harbor IDH1 mutations contain IDH2 mutations [24]. Careful genomic rescoring of tissue bank specimens demonstrates that virtually 100% of 1p/19q whole-arm-lost oligodendrogliomas harbor *IDH* gene mutations [39]. Thus, for class definition, it may be reasonable to require the presence of *IDH* gene mutations to identify oligodendroglioma, and their absence may indicate alternative histologic ‘mimics’ (such as DNET or clear-cell ependymoma [37]). Conversely, it has been recognized for some time that *TP53* mutations are commonly found in grade II and III astrocytic gliomas, but are rarely found in oligodendroglial tumors [40]. Such molecular analyses call into question the histopathologic identification of a ‘mixed’ oligoastrocytoma as a discrete entity, as opposed to cases with histologic manifestations drawn from an overlapping spectrum of two otherwise distinct molecular groups: 1p/19q-codeleted oligodendroglioma and *TP53*-mutant astrocytoma. This inverse relationship proves to be even sharper within the *IDH*-mutant subgroup of gliomas [41].

These clear-cut divisions allowed for the emerging outlines of a class-defining description of three ‘molecular groups’ of diffuse gliomas corresponding in large part to major histopathologic entities: *IDH* wild-type gliomas (characterizing the vast majority of primary glioblastomas), *IDH*-mutant, *TP53*-mutant astrocytic gliomas (consisting of lower- and intermediate-grade astrocytomas, and progressive/secondary glioblastomas) and *IDH*-mutant 1p/19q-codeleted oligodendroglial gliomas (consisting of mostly ‘pure’ oligodendrogliomas) [19] (Figure 1).

• CIC/FUBP1

The acceleration of genomic sequencing technology then rapidly facilitated further ‘class-defining’ mutation identification. Focused analysis of 1p/19q-codeleted oligodendrogliomas resulted in the identification of inactivating mutations in the *CIC* gene on chromosome 19 and the *FUBP1* gene on chromosome 1p [42–44].

Figure 1. Concurrent molecular genetic alterations identify glioma subgroups. Three groups of adult diffuse gliomas are identified, each characterized by distinct and largely non-overlapping molecular genetic features. The most common group is typically found in older patients, with TERT promoter mutation in the absence of *IDH* gene alterations, and characteristic “glioblastoma” histology. The second most-common is found in younger adults, with concurrent *IDH*, *TP53* and *ATRX* alterations and astrocytic histology. The least common, oligodendroglial tumors, are found in patients between the two extremes of age, with *IDH* and TERT promoter mutations, along with 1p/19q-codeletion. Oligodendroglioma and glioblastoma, to a lesser degree, have been proven to derive clinical benefit from treatment with chemotherapy. Astrocytic tumors do not have a similar evidence base.

CIC, which is most frequently found to be mutated in these tumors, is thought to function downstream of growth factor receptor signaling pathways by binding to DNA regulatory elements as a reversible repressor of target genes [45]. Notably, missense mutations in *CIC* in oligodendrogliomas cluster within the putative DNA binding domain. This tumor-suppressor spectrum of *CIC* mutational inactivation suggests that oligodendrogliomas may have a basal level of ‘released’ activation of these pathways, which might explain why growth factor receptor amplification events are rarely observed in this tumor type, as they might not provide additional selective growth advantage for the tumor cells.

FUBP1 is a little-studied DNA-binding protein, with unknown function in gliomagenesis.

The relationship between *CIC/FUBP1* inactivation and chemosensitivity, if any, is yet unknown and awaits further study.

• *ATRX/TERT*

Additional studies have identified mutations in the *ATRX* gene on the X chromosome in *IDH*-mutant astrocytic gliomas [19,46–48]. *ATRX* is a large protein that complexes with histones and heterochromatin at centromeres and telomeres, whose inactivation is associated with the alternative lengthening of telomeres (ALT) phenotype. As mentioned above, *IDH*-mutant astrocytic gliomas are typically *TP53* mutant, and *ATRX* inactivation therefore serves as an additional identifying genetic alteration to positively categorize these lesions, and exclude a

diagnosis of oligodendroglioma. Since immunohistochemical staining of ATRX can be used to identify inactivation of this protein [49], retained nuclear staining of ATRX in a tumor positive for IDH R132H immunohistochemical staining and the histologic appearance of oligodendroglioma offers a confirmatory molecular diagnostic approach to identify 1p/19q-codeleted, IDH-mutant, CIC-mutant oligodendroglioma.

Recent work has also identified *TERT* promoter somatic mutations in IDH-mutant, CIC-mutant oligodendrogliomas [50]; these mutations are associated with overexpression of TERT [51]. Interestingly, somatic *TERT* promoter mutations are also found in the vast majority of IDH wild-type glioblastomas, while they are virtually never seen in IDH-mutant TP53-mutant ATRX-mutant astrocytic gliomas. Thus, for the diffuse gliomas, *TERT* mutations and ATRX mutations are mutually exclusive. Oligodendrogliomas, therefore, now are recognized to contain at least four different recurrent molecular genetic correlates: IDH mutation, 1p/19q loss, CIC mutation and *TERT* promoter mutation.

Epigenetics

Epigenetic alterations are changes that affect cell behavior through events other than direct DNA sequence changes such as mutations and amplifications. Examples include nonsequence modifications to DNA itself, such as DNA methylation, as well as changes in DNA-related proteins, such as post-translational modification of histones. Because much of the control of DNA expression is mediated at an epigenetic level, epigenetic alterations can have tumorigenic effects, including in gliomas [52]. In adult glioblastoma, for example, methylation of the MGMT promoter occurs in approximately half of cases and is associated with improved response to alkylating agent chemotherapy [53]. In pediatric glioblastomas, mutations occur in histone genes (*H3F3A*) or in genes that regulate histones (*ATRX* and *DAXX*), implying that deregulation of histone function is critical to pediatric gliomagenesis [54]. As mentioned above, mutation of the IDH genes appears to be a causative factor in the global DNA methylation phenotype in gliomas known as the glioma-CpG island methylator phenotype (G-CIMP) [29,30].

Few studies have explored epigenetic alterations in oligodendrogliomas. MGMT promoter methylation has been documented in many oligodendrogliomas [55] and hypermethylation

can be found in the promoter regions of many tumor-related genes in oligodendrogliomas [56]. A subset of oligodendrogliomas have widespread changes in DNA methylation akin to G-CIMP [57]. Since IDH mutations are near-universal in oligodendrogliomas, extensive methylation abnormalities in these tumors may be related to their mutant IDH status.

It therefore remains possible that some of the biological effects reflected in 1p/19q loss and the associated molecular genetic alterations such as IDH1 mutation result from alterations in epigenetic regulatory mechanisms, similar to what has been noted in other tumor types in which pathogenic changes may be related to promoters, regulatory enhancers and noncoding lincRNAs [52]. For example, loss of distal enhancers on 1p/19q could lead to changes in transcriptional regulation via loss of interactions between regulatory sites and target promoters, which could account for the tumor-promoting phenotypes seen with methylation of the *NHE-1* gene on chromosome 1 [58]; similarly, the 1q/19p fusion could create novel interactions between enhancers and promoters. At the present time, however, these possibilities have not yet been evaluated in oligodendrogliomas.

Modern management & future directions

The clustering of recurrent molecular abnormalities within these groups has allowed for diffuse glioma diagnosis based on a consensus of identifiers, or ‘molecular group’ (Figure 1). While each molecular test has imperfect sensitivity and specificity, the tendency for overlap with other mutations can be combined with histologic information and clinical/demographic details to draw the borders between these tumor categories more sharply. Indeed, these diagnostic pursuits drive the modern management of a patient at initial presentation with a diffuse glioma. A firm consensus ‘molecular group’ diagnosis provides a stronger rationale for the subsequent adjuvant treatment of patients, allowing clinicians to use a more evidenced-based approach. It is tempting to envision molecular diagnostic approaches that will score for the two complementary hotspot recurrent mutations (*IDH1* and *TERT*) to facilitate rapid and specific glioma subclassifications on even small biopsy specimens.

Still, there remain many areas of ongoing uncertainty, and prior studies can be reinterpreted with an eye toward molecular groups, to highlight the areas in need of a strengthening evidence base to guide treatment. As a critical first step, molecular

retrospective review of previous glioma studies could be undertaken with the above-described immunohistochemical and PCR-based sequencing techniques, which are amenable to efficient scoring of formalin-fixed paraffin-embedded tissues from clinical trial specimen banks.

For oligodendrogliomas, such a re-examination of the RTOG and EORTC studies outlined above suggests that adjuvant PCV, when combined with radiation therapy, provides a substantial survival benefit to the 1p/19q-codeleted, *IDH*-mutant, *CIC*-mutant subgroups. It is possible that the administration of PCV chemotherapy following radiation therapy to patients with grade II oligodendrogliomas with 1p/19q codeletion, *IDH* mutation and *CIC* mutation may provide similar clinical benefit.

The data emerging from the ongoing grade II glioma study RTOG 9802 suggest this 'molecular grouping' perspective may be appropriate. In outcomes recently announced, patients with grade II gliomas (defined as high risk – either patient aged >40 years, or subtotal resection), PCV chemotherapy in addition to radiation therapy was associated with a median survival extension to 13.3 years from 7.8 years. Intriguingly, the patients in this study were not molecularly stratified, and it would not be expected that even the majority of the tumors were 1p/19q-deleted oligodendrogliomas – for

instance, in the 'low-risk' observation arm of this same study, only 50 of 111 patients were categorized as 'pure oligodendroglioma' histologically [59].

These results raise the possibility that the mechanistic driver of PCV responsiveness may be the presence of *IDH* mutation, which is common to both oligodendroglial and astrocytic low-grade gliomas. A recent molecular reanalysis of the RTOG 9402 data may support this hypothesis, as AO and AOA patients having an *IDH* mutation derived a substantial survival benefit from PCV chemotherapy [60]. However, similar analyses of patients from the EORTC 26951 study did not reveal that *IDH* mutation was predictive in an otherwise histologically similar cohort [61,62]. It is hoped that future appropriately designed clinical trials, with attention to molecular genomic alterations, will resolve these questions.

Financial & competing interests disclosure

This work was supported by the Burroughs–Wellcome Career Award (DPC). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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