Can molecular markers help with decision making?

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WHO classification system remains unsatisfactory due to its lack of reproducibility [1] and lack of precision in terms of prognosis. Tumors that share identical histopathological features can actually represent multiple distinct molecular phenotypes. Several molecular markers provide prognostic information and may help in decision when providing predictive information.

Early-retrospective studies suggested that the codelletion of chromosome 1p and 19q was a predictor of response to chemotherapy and longer progression-free survival in anaplastic oligodendrogliomas [2]. In both low-grade and anaplastic oligodendrogliomas, 1p19q codeletion was associated with longer progression-free survival and overall survival whatever the treatment [3–5]. Until recently, it was still unclear whether 1p19q loss was predictive for chemotherapy or merely indicates a different natural history [6, 7]: recent RTOG (Radiation Therapy Oncology Group) 9402 and EORTC 26951 update shows unambiguously near-doubling of median survival times (14.7 versus 7.3 years in the RTOG study) of patients with 1p19q codeleted grade III gliomas treated with chemotherapy and radiation therapy (RT) versus RT alone, whereas patients without codeletion have a poor survival (2.6–2.7 years) and no substantial benefit of adjuvant chemotherapy (G. Cairncross and M. van den Bent, unpublished data). The 1p19q codelleted gliomas overexpress proneural genes: one of them is INA which encodes α-internexin (INA). Immunohistochemical INA analysis can be used routinely and is a simple and valuable prognostic and predictive factor for adjuvant chemotherapy [8–10].

The recurrent IDH1 mutation is restricted to the 132 residue of the IDH1 gene, the majority (>90%) being a CGT → CAT change, leading to an Arg132 → His substitution [11]. Some patients without IDH1 mutations harbor a mutation in the analogous amino acid residue (Arg132) of the mitochondrial isoform IDH2 [12]. IDH1/IDH2 mutation is inversely correlated with grade, affecting nearly 3/4 of WHO grade 2, half of WHO grade 3 gliomas, and only 5% of primary glioblastomas [13], whereas 80% of secondary glioblastomas are IDH1/IDH2 mutated [14]. IDH1/IDH2 mutations are extremely rare in non-glial malignancies and infrequent in pilocytic astrocytomas [12, 15]. IDH1/IDH2 mutational status is therefore also a useful diagnostic marker of glioma versus other intracranial tumors and may help to differentiate grade 2 gliomas from pilocytic astrocytomas, or secondary glioblastomas from primary glioblastomas [16]. This is considerably facilitated by the development of two monoclonal antibodies specifically targeted against the IDH1 R132H mutation [17, 18] and, above all, by non-invasive determination of IDH1/IDH2 mutational status, such as 2-HG dosage by Spectro-magnetic resonance imaging [19, 20].

IDH1/IDH2-mutated tumors have a better outcome, whatever grade considered [13, 14, 21]. Whether IDH1/IDH2 mutation can predict response to treatment in gliomas needs to be further investigated [22].

The mutation causes the loss of the isocitrate dehydrogenase function and the gain of an α-ketogluturate reductase function leading to the cellular accumulation of D-2-hydroxyglutarate (D-2HG). The rate of D-2HG in IDH1-mutated tumors is increased by a factor of >100, thus representing a diagnostic marker (this change is almost specific for gliomas) and prognostic (mutated gliomas have longer survival) of interest. The combination of 2-HG is directly involved in histone and DNA methylation. Interestingly IDH1/IDH2 mutations are tightly associated with genetic profile (IDH1 or IDH2 mutation is a constant feature in 1p19q-codeleted gliomas [23]) and with the methylation of MGMT promoter, and more generally with a methylation profile [24, 25].

MGMT removes alkyl groups from the O6 position of guanine [26] and is supposed to limit the effectiveness of alkylating agents [27]. Epigenetic silencing of the MGMT gene by promoter methylation is associated with the loss of MGMT expression [26]. Based on the initial studies in glioblastomas and on its biological role, MGMT promoter methylation has been mostly considered as a predictive factor of response to chemotherapy. In fact, recent studies showed that MGMT promoter methylation is also predictive of better response to radiotherapy, suggesting that it may be a marker of better therapeutic response [28].

In glioblastomas, the EORTC/NCIC 26981/22981 clinical trial showed a clear benefit of concomitant and adjuvant temozolomide (TMZ) to RT for patients with MGMT promoter methylation (overall survival = 27.2% at 2 years, 16.0% at 3 years, and 9.8% at 5 years versus 10.9%, 4.4%, and 3.0% for patients receiving radiotherapy alone) but not for
patients without MGMT promoter methylation despite a higher probability of long-term survival [26, 29]. The prognostic impact of MGMT methylation status has been also investigated in gliomas of lower grades. Surprisingly, MGMT promotor methylation was reported to be prognostic but not predictive in anaplastic oligodendrogial tumor patients treated with radiotherapy and adjuvant packed cell volume versus radiotherapy alone [30]. Similarly, the NOA-04 trial reported an important difference in progression-free survival among patients with or without MGMT promoter methylation, even when treated with radiotherapy alone [21], suggesting that MGMT methylation reflect a diffuse methylation profile the CIMP profile (CpG Island Methylated Phenotype) known to be of better prognosis (see below). Similarly, methylation is also a prognostic factor in low-grade glioma patients treated by temozolomide (TMZ) [31]. Several techniques for the determination of the MGMT promoter methylation status are available, including methylation-specific PCR (MSP), semi-quantitative MSP, and pyrosequencing [32]. Clearly, the standardization of MGMT testing requires further comparison of different technologies across laboratories and prospectively validated cutoff values for prognostic or predictive effects.

The relation between MGMT promoter methylation and MGMT expression is complex. Despite statistical agreement between MGMT promoter methylation and MGMT expression, a substantial rate of discordance still persists and some CpG regions better reflect MGMT expression than do others [32]. It is still not clear whether transcriptional repression is the key mechanism that explains the higher chemosensitivity of promoter methylated tumors. Strikingly, MGMT expression assessed using immunohistochemistry did not correlate with the outcome in a study on 184 patients included in the EORTC/NCIC 26981/22981 trial. In fact, it is possible that the favorable outcome associated with MGMT promoter methylation might not only be related to MGMT down-regulation but may also reflect a more global pattern of epigenetic alterations that could explain both responses to chemotherapy and to radiotherapy. Interestingly, a subset of gliomas characterized a wide pattern of CpG methylation and more favorable outcome has been identified [33]. These caveats should lead clinicians to be cautious when deciding on a therapeutic strategy based on MGMT methylation status alone. However, because it is an important prognostic and predictive factor in gliomas, ongoing clinical trials use MGMT promoter methylation status to stratify patients (e.g. the EORTC 26053–22054 trial in anaplastic gliomas) and even to select them (e.g. the NCT00813943 trial in glioblastomas).

EGFR (epidermal growth factor receptor) amplification involves 40% of glioblastomas and is associated in 25% of the cases with the deletion of the exons 2–7 resulting in a truncated, constitutively active protein (EGFRvIII). EGFR amplification has no clear prognostic impact on glioblastoma. However, when it is present in grade 3 or 2 (rarely), it is predictive a poor outcome: such tumors should be considered and treated as a glioblastoma. EGFR amplification is never associated with 1p/19q codeletion and extremely rarely with IDH1/IDH2 mutations. Large-scale gene expression profile studies have shown that transcriptional profiles reflect the underlying tumor biology and can be used to predict tumor classification, patient outcome, and response to treatment. Furthermore, specific genetic changes, EGFR amplification, IDH1 mutation, and 1p/19q codeletion segregated in distinct molecular subgroups [34]. The Cancer Genome Atlas (TCGA) consortium established a robust transcriptomic classification of glioblastomas, into four classes called classical, mesenchymal, proneural, and neural. IDH1 mutations are found exclusively in the proneural, whereas EGFR amplification is almost constant in the classical subtype [35]. Taken together, these data strongly support the diagnostic/prognostic classification based on 1p19q, IDH1/2, and EGFR status, as a valuable complement of WHO classification. In addition, several studies have proposed multigene signatures predictive of outcome: based on the analysis of four independent data sets, Colman et al. identified a robust nine-gene signature that determines two groups of gliomas with radically different prognosis, independently from clinical factors and MGMT promoter methylation status. Interestingly, this multigene predictor is applicable to routinely processed, formalin fixed-paraffin embedded samples [36]. Based on the gene expression profile of a large series of 276 gliomas (grades 1–4), Gravendeel et al. identified robust clusters of gliomas and found that the gene expression profile of gliomas was a better predictor of the outcome than histology. Again, specific genetic changes (EGFR amplification, IDH1 mutation, and 1p/19q codeletion) segregated in distinct molecular subgroups [34]. De Tayrac et al. [35] conducted a meta-analysis on three publicly available microarray data set and identified a risk score based on the expression of four-gene and strongly associated with the outcome of patients with high-grade gliomas [37].

**disclosure**

The author has declared no conflicts of interest.

**references**


