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ABSTRACT

Introduction: Mutations in the genes isocitrate dehydrogenase (IDH) 1 and 2 have been reported in a limited number of tumors. In gliomas, IDH mutations are primarily detected in WHO grade II–III tumors and represent a major biomarker with diagnostic, prognostic, and predictive implications. The recent development of IDH inhibitors and vaccines suggests that the IDH mutation is also an appealing target for therapy.

Areas covered: This review focuses on the role of IDH mutations in diffuse gliomas. Besides discussing their role in gliomagenesis, we will emphasize the role of IDH mutations in clinical practice as a diagnostic, prognostic and predictive biomarker, and as a potential therapeutic target. Noninvasive detection of the IDH mutation by means of liquid biopsy and MR spectroscopy will also be discussed.

Expert commentary: While IDH mutation is a consolidated diagnostic and prognostic biomarker in clinical practice, its role in oncogenesis is far from being elucidated, and there are several pending issues. The routine use of noninvasive techniques for detection and monitoring of the IDH status remains challenging. Although the IDH mutation is a very early alteration in gliomagenesis, it may then be omitted during tumor progression. This observation has important implications when designing targeted clinical trials.

KEYWORDS

IDH1and IDH2 mutations; gliomagenesis; grade II and III gliomas; biomarkers; target therapy

1 Introduction

Isocitrate dehydrogenase (IDH) mutations are hotspot mutations affecting the genes that encode for the isocitrate dehydrogenase 1 (*IDH1*, cytoplasmic isoform) or 2 (*IDH2*, mitochondrial isoform) enzyme, all resulting in amino acid substitution at the active site and finally in a neomorphic activity that leads to profound modifications in the epigenetic profile of the cell.

IDH mutations have been first identified in diffuse gliomas in 2009 [1,2]. Since their discovery, IDH mutations have acquired an increasingly prominent role in diffuse gliomas management, as they bear diagnostic, prognostic, and predictive implications. They now represent the cornerstone of glioma classification and prognostic stratification, and they are actively investigated as a potential actionable target.

Mutations in the IDH genes have subsequently been identified in other tumor types, including acute myeloid leukemia ($IDH1^{R132}$, $IDH2^{R140}$, and $IDH2^{R172}$) [3], cholangiocarcinomas ($IDH1^{R132}$ and $IDH2^{R172}$) [4], chondromas and chondrosarcomas ($IDH1^{R132}$ and$IDH2^{R172}$) [5], angioimmunoblastic T-cell lymphoma ($IDH2^{R172}$) [6], melanomas ($IDH1^{R132}$) [7], and a rare subtype of breast cancer (solid papillary carcinoma with reverse polarity, $IDH2^{R172}$) [8]. Clinical trials on IDH inhibitors are ongoing in these populations as well.

The present review will focus on the role of IDH mutations in diffuse gliomas. Besides analyzing the role of IDH mutations in gliomagenesis, we will discuss the use of IDH mutations in clinical practice as a diagnostic, prognostic, and predictive biomarker, as well as an actionable target. Emerging techniques for noninvasive detection of the IDH status will be described, with reference to their limitations and benefits in daily practice. Lastly, perspectives and future developments will be illustrated.

1.1 IDH mutation, D2HG production, and gliomagenesis

IDH mutations concern most of lower (i.e. II and III) grade gliomas and secondary glioblastomas, and only 5% of – apparently – primary glioblastomas [9].

Mutations in the IDH genes affect the codon 132 of IDH1 ($IDH1^{R132}$, in about 90% of cases) or the codon 172 of IDH2 ($IDH2^{R172}$, in about 10% of cases) [10], in a mutually exclusive way. In both cases, the hotspot mutation results in amino acid substitution at a key residue of the encoded protein. $IDH2^{R140}$ mutations, common in acute myeloid leukemia [11], have not been reported in gliomas [12]. Mutations arising in $IDH1^{G97}$ [13,14], $IDH1^{R100}$ [14–16] (the homologous of $IDH2^{R140}$), $IDH1^{R109}$ [17], and $IDH1^{R314}$ [18] have been anecdotally reported in gliomas.

IDH1 and IDH2 are enzymes that physiologically convert isocitrate to α-ketoglutarate (α-KG). These two enzymes have similar function but different cell compartmentalization: IDH1 is located in the cytosol, while IDH2 in mitochondria. Mutations in either IDH1 or IDH2 result in a neomorphic enzymatic activity, causing the stereospecific conversion of α-KG

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(produced by the wild type enzyme) to the D-enantiomer of 2-hydroxyglutarate (D2HG), that in turn accumulates within tumor cells [19,20]. IDH mutations are virtually always heterozygous mutations. The presence of a wildtype copy of the gene is necessary to provide the substrate for the mutant enzyme to produce the D2HG [21]. Loss of the wildtype allele, which may occur during tumor progression, also reduces the neomorphic activity [22].

D2HG acts as an oncometabolite and it is supposed to be the main driver of gliomagenesis. Because of its structural affinity to α-KG, D2HG competitively inhibits several α-KG-dependent dioxygenases (as the JmjC domain-containing family of histone demethylases [23–25] and the TET family of methylcytosine hydroxylases [25,26]) with profound consequences on the epigenetic regulation of the cell. The enzymatic blockade induced by the accumulation of D2HG results in increased levels of histone H3 lysine methylation levels [24] and in global DNA hypermethylation (glioma CpG island methylator phenotype, G-CIMP) [26–28]. Of note, histone H3.3 mutations, frequently found in pediatric gliomas [29,30], and TET2 mutations, frequently found in myeloid malignancies [31], are mutually exclusive with IDH mutations [26,29,30,32], suggesting that they have overlapping roles in tumorigenesis. The recently developed DNA methylation-based classification of central nervous system tumors [33] clearly shows that IDH mutant gliomas represent a well-defined methylation class, strongly separated from the nonmutant counterpart. In TGCA gene expression-based molecular classification of glioblastomas, IDH mutation strongly relates with the Proneural subtype [34].

Of interest, two different inborn error of metabolism can cause the pathological accumulation of one of the 2HG enantiomers, conditions called respectively D- and L-2HG aciduria. Strikingly, an increased risk of malignant brain tumors has been reported for L2HG aciduria [35–37], but not for D2HG aciduria [38].

While IDH mutations profoundly modify gene expression, a direct oncogenic effect has not been clearly demonstrated. Induced expression of $IDH1^{R132H}$ mutations in CNS cells of mouse models failed to induce glioma formation [39,40]. IDH mutation and the subsequent epigenetic remodeling result in a block of differentiation [24] that acts as a fertile substrate in which, over time, the occurrence of new 'lineage-defining' alterations (i.e. 1p/19q codeletion, CIC, FUBP1, and pTERT mutations in oligodendrogliomas; p53 and ATRX mutations in astrocytomas) lead to gliomagenesis [41–43]. Succeeding 'tertiary' alterations (activating mutations of intracellular signaling pathways or amplification/activation of receptor tyrosine kinases) may then occur and lead to tumor progression to higher grades [44].

It is likely that the IDH mutation is not needed along the whole natural history of the tumor. At the latest stages of the disease, IDH mutation is no longer a driver alteration for a large number of gliomas, and becomes a passenger mutation while tertiary driver alterations appear. Johannesen et al. [45] . showed that the IDH mutant enzyme is not required for tumor maintenance after the occurrence of tertiary oncogenic alterations. Consistently, Mazor et al. [46] identified in recurrent gliomas IDH1 copy number alterations (CNA) resulting in impaired D2HG production. *In vivo* and *in vitro* models showed clonal selection for CNA, suggesting an advantage from losing IDH mutant function once tertiary alterations are established [46]. These findings suggest a potential benefit from loss of mutated function in advanced stages of IDH mutant gliomas. These observations still need further confirmation but could affect the development of target therapies directed to IDH mutant blockade.

Recent studies added further elements to this scenario, showing that the IDH mutation-driven methylator phenotype can directly produce oncogenic signals by reshaping the three-dimensional (3D) chromatin structure. Flavahan et al. [47] showed that DNA hypermethylation in specific sites reduces its binding with CCCTC-binding factor (CTCF), an insulator protein, modifying therefore the 3D structure of the chromatin; this leads to disruption of genome topological domain boundaries. PDGFRA, an important oncogene, was aberrantly activated through interaction with a constitutive enhancer physiologically relegated in a different domain [47]. Similarly, in IDH mutant astrocytes, the repression of SOX2, a condition that acts as an early driver of gliomagenesis, is due to the hypermethylation of DNA binding sites for the chromatin insulator CTCF, leading to the dissociation of the SOX2 promoter from critical enhancer elements [48]. These two significant examples illustrate the pleiotropic and complex effects of IDH mutation on gene regulation.

The D2HG released in the microenvironment may also alter the functioning of peritumoral non-neoplastic cells, such as neurons and immune cells. The presence of IDH mutation has been linked with an increased risk of seizures in gliomas [49]. D2HG is structurally similar to glutamate and activate NMDA receptors in *in vitro* models [50,51], and may cause abnormal discharges in infiltrated neurons. More importantly, recent works have shown that IDH status also modulates the tumor-associated immune system. Compared to their wildtype counterpart, IDH mutant gliomas show reduced immune infiltrates [52–55] for both CD4 + and CD8+ lymphocytes [55]. It has been first speculated that this could in part explain the better outcome of IDH mutant gliomas, as immune infiltration is generally linked to poorer outcome [56] 8. In fact there are now direct evidences showing that D2HG accumulation exerts immunosuppressive effects. Activated CD4+ and CD8 + T cells proliferation is decreased in presence of high level D2HG [55]. D2HG reduces both the expression of several chemokines (such as CXCL10) and T cells migration in presence of chemoattractants, leading to a reduced immune infiltration [52,54,55]. Importantly, this immunosuppressive effect is reversed by IDH inhibitors. Moreover, the D2HG accumulation impairs complement-mediated tumor cell killing with both classical and alternative pathways [55]. IDH mutant tumors can also acquire mechanisms of resistance to natural killer cells by silencing the expression of the NKG2D-ligands ULBP1 and ULBP3 [57]. Interestingly, this could be antagonized by decitabine, an hypomethylating treatment which *in vitro* restored expression of NKG2D ligands. Very recently it has been shown that D2HG released by tumor cells is taken up by T cells and impairs their function. D2HG disrupt transcription and nuclear translocation of nuclear factor of activated T cells and promotes polyamine synthesis, finally resulting in reduced ATP/ADP levels and thus suppression of T cell activity and proliferation [58]. All these mechanisms contribute to severely affect the antitumor immune response. On the other hand, the expression of the immune checkpoint protein PD1, which is an important mechanism of tumor escape [59], is

repressed in IDH mutant gliomas by promoter methylation. This may participate to the resistance to immune checkpoint inhibitors treatments [60].

It is getting also more and more likely that not all the IDH mutation effects are mediated by the D2HG. The activity of IDH mutant enzyme leads to reduced levels of NADPH [61,62]. Seen its prominent role in protection from reactive oxygen species, this metabolic alteration may explain the enhanced radiosensitivity of IDH mutated gliomas.

1.2 The diagnostic and prognostic role of IDH mutation in diffuse gliomas

There is now solid evidence that IDH mutations have a major diagnostic and prognostic role in diffuse gliomas [9,63–67]. On these bases, the IDH status has been incorporated in the 2016 revision of the WHO classification of Tumors of the Central Nervous System as a defining feature for different tumor entities [68]. The codeletion of chromosomes 1p and 19q, that invariably associates with the IDH mutation [63], is the other molecular alteration included into the classification of diffuse gliomas. The combination of the IDH and 1p/19q codeletion status allows to distinguish three distinct molecular subgroups: (1) IDH mutant, 1p/19q codeleted gliomas (corresponding to grade II and III oligodendrogliomas); (2) IDH mutant, 1p/19q non-codeleted gliomas (corresponding to grade II and III astrocytomas and secondary glioblastomas); (3) IDH wildtype gliomas (primary glioblastomas and a minority of diffuse grade II and III astrocytomas). The prognostic stratification based on these molecular subgroups accounts better than the classification based on the histological appearance alone for the biological behavior of the neoplasm [65,69]. IDH mutant, 1p/19q codeleted gliomas harbor the better prognosis, with a median survival of 15 years for both grade II and III. IDH mutant, non-codeleted gliomas have a median survival of 7–9 years for grade II and 5–6 years for grade III. IDH wildtype lower grade gliomas have the poorest prognosis, approaching glioblastoma median survival (2 years for grade III, 3.5 years for grade II) [63,66,67].

While grading (II vs. III) has a modest impact in IDH mutant gliomas [70], the loss of CDKN2A/B on 9p21, the presence of necrosis and for astrocytomas the total number of copy number variation are independent prognostic factor and may be used to define an algorithm that is superior to histological grading in predicting the overall survival [71–73].

The IDH mutation also predicts the response to cytotoxic treatments. IDH mutant gliomas are more sensible to radiotherapy, as they have reduced levels of NADH and NADPH [61,62]. The pharmacological inhibition of IDH restores NADH and NADPH levels and reduces the cytotoxic effect of ionizing radiations on tumor cells [62]. These data suggest that the administration of IDH inhibitors during radiotherapy might decrease the cytotoxic effects of radiation.

IDH mutant tumors also display improved response to chemotherapy: randomized clinical trials conducted in patients with high risk grade II [74] and grade III [75,76] gliomas treated with radiotherapy showed that only patients with IDH mutant tumors clearly benefit from the addition of adjuvant PCV (procarbazine-CCNU-vincristine) chemotherapy to radiation. IDH mutant gliomas have a reduced expression of O6-methylguanine DNA methyltransferase gene, a known DNA repair enzyme associated to chemosensitivity [77], via promoter hypermethylation [28]. Moreover, D2HG competitively inhibits several αKG-dependent dioxygenases involved in DNA repair, as the AlkB family [78,79] and KDM4A/B enzymes [80]. All these observations may explain the higher sensitivity of IDH mutant tumors to alkylating agents.

1.3 IDH status assessment: immunohistochemistry and sequencing

The IDH genotype is assessed by sequencing of tumor DNA extracted from fresh frozen or formalin-fixed paraffin-embedded tissue. The most common sequencing technique to assess the IDH status remains the Sanger method, as it is a widely used and low-cost technique. However, Sanger sequencing can miss IDH mutations when mutant allele levels are below 15–20%; this primarily occurs when the tumor sample is heavily contaminated by normal cells, as it occurs in peripheral surgical sampling.

Pyrosequencing is a quantitative DNA sequencing technique based on the detection of pyrophosphate released during nucleotide incorporation ('sequencing by synthesis') that can identify IDH1/2 mutant allele frequency as low as 5% [81,82]. Real-time PCR with post-PCR fluorescence melting curve analysis also showed higher analytical sensitivity compared to Sanger sequencing [83].

Newer sequencing techniques with higher sensitivity have been developed during the last few years. PCR clamping/Amplification Refractory Mutation System [84] and CO-amplification at Lower Denaturation temperature PCR (COLD-PCR) [85] are based on the preferential amplification of the mutant allele and may detect mutant IDH allele levels below 5%. Droplet digital PCR [86] relies on DNA partitioning in nanoliter-sized droplets, which then carries out a PCR reaction with a single-molecule resolution.

The IDH status can alternatively be assessed using multiplexed, high-throughput genotyping platforms [87,88], and deep next generation sequencing panels [89–92]. These panels allow the contemporary acquisition of a wide panel of genetic alterations, including mutations, amplifications, and deletions.

As IDH mutant tumors express a DNA hypermethylator profile [27,28], IDH status can be inferred from tumor DNA profiling on 450 and 850k arrays.

Immunohistochemistry is routinely performed as part of the histopathological assessment. As mutations in the IDH1 gene consist in more than 90% of cases in an arginine-to-histidine substitution at residue 132 [10][9], the use of a specific antibody that binds the mutant IDH1^{R132H} protein allows to identify mutant tumor cells by immunohistochemistry in most cases [93,94]. This method can identify single mutated cells over a background of normal cells, with a sensitivity that may overcome standard sequencing [95]. However, non-R132H IDH1 mutations and IDH2 mutations are missed. In case of immunohistochemistry negativity and with the suspicion of an IDH mutation based on patient age (patients with glioblastomas younger than 55 years) and/or

histological features (e.g. astrocytoma phenotype, loss of ATRX expression, oligodendroglial phenotype, or 1p19q codeletion), IDH1 and IDH2 sequencing should be performed. Sequencing can be omitted in case of elderly patients (i.e. >55 years old) with an histological appearance of glioblastoma, or an immunostaining positivity for the H3K27M mutation, as the two genetic alterations are known to be mutually exclusive [29].

1.4 Noninvasive detection and monitoring of the IDH mutations

Further from well-defined IDH testing from tumor specimen, there is a strong need for less-invasive testing, for several reasons: *in primis*, not all gliomas are amenable to resection, and the identification of IDH mutation could permit the diagnosis without the surgical risk of a biopsy; furthermore, all biopsies are burdened by the possibility of sampling error, and could underscore the IDH status or even the glioma diagnosis; the identification of a quantifiable biomarker could eventually help in evaluating the response to therapies, in differential diagnosis with pseudoprogressions and pseudorelapses, and in predicting relapses before the neuroimaging. Lastly, it has been suggested that the IDH mutation drive the prognostic benefit associated with a greater extent of resection [96], so knowing the IDH status would be of interest also in the preoperative planning. On this regard, PCR [97,98]-, desorption electrospray mass spectroscopy [99,100], matrix-assisted laser desorption ionization-time of flight mass spectroscopy [101], and optical spectroscopy [102,103]-based techniques have been developed for rapid detection of IDH mutation or D2HG accumulation in the intraoperative setting.

A promising biomarker for IDH mutant tumors is D2HG, as it is produced at detectable concentrations in tumor tissues only [19], and its level has been correlated with response to IDH inhibitors [104] in preclinical models.

Tumor D2HG millimolar levels can be detected *in vivo* with magnetic resonance spectroscopy [105–107]. Identification of the metabolite is diagnostic for IDH mutation, as it is absent in normal brain tissue. However, standard PRESS sequences are burdened by up to 26% of false positive due to overlapping with the signal from other metabolites of normal brain (as glutamate and glutamine) at spectrum location of 2.25 ppm [107]. Several techniques have been experimented to overcome the limit and optimize D2HG detection, as TE 97 ms optimized PRESS [106], 2D correlation spectroscopy [105], and optimized semi-LASER [108] (see Leather et al. [109] for review). Edited MRS (based on detection of D2HG signal at 4.02 ppm, using MEGA-PRESS sequence) recently showed being more reliable than optimized PRESS MRS in an extended series of patients, reaching an optimal 100% sensitivity, specificity, PPV, and NPV for diagnosis [110].

Two series evaluated D2HG-detecting MRS (with optimized PRESS sequences) in a clinical setting, with different results in terms of sensitivity (48–89.5%) and specificity (81.3–100%) [111,112]. Sensitivity appeared to be mostly influenced by tumor dimension [111], while low specificity were attributed to inclusion of necrotic areas [112], which presence is however uncommon in IDH mutant gliomas and should raise the suspicion of a false positive result. One may speculate that this may be due to the production of L2HG by hypoxia [113], as spectroscopy technique is unable to differentiate the two enantiomers.

Other than diagnostic, D2HG-detecting MRS can also be helpful in longitudinal follow up, as D2HG signal decrease in correlation with treatment response [111,114,115].

As D2HG-detecting MRS is not widely diffused, other papers explored the utility of standard and advanced MRI techniques in predicting IDH status. IDH mutant gliomas usually are better defined, poorly or not enhancing, lobar tumors, while IDH wildtype gliomas present a greater percentage of non-lobar and multifocal locations [116]. In diffusion and perfusion sequences, IDH mutation is associated with higher apparent diffusion coefficient and lower regional cerebral blood volume values [117–119]. The 'T2-FLAIR mismatch' sign (i.e. a completely hyperintense signal in T2 sequences corresponding to an hypointensity with hyperintense rim in fluid attenuated inversion recovery [FLAIR] sequences) is highly specific, though less sensitive, for IDH mutant, 1p/19q codeleted tumors [120,121, 122].

Another promising tool for poorly invasive prediction of IDH status is the detection of D2HG levels in body fluids. Differing from patients with AML [123] and cholangiocarcinoma [124] harboring IDH mutations, studies failed to find a detectable elevation of D2HG in serum of patients with IDH mutant gliomas [125–127]; this may be attributed to the site-specificity of D2HG production. A further paper examined the D2HG levels in CSF and found a good specificity in detecting IDH mutation; however, the data were stronger for necrotic, contrast-enhancing higher grade gliomas compared to LGGs which represent the majority of IDH mutant gliomas [128]. Urinary D2HG levels in IDH mutant and wildtype gliomas have been also analyzed, but with discordant results [126,127].

A progressing field in oncology is the so called 'liquid biopsy,' which aims to detect tumor cells or tumor nucleic acids in body fluids [129,130]. Highly sensitive techniques as digital droplet PCR [131] or COLD PCR [85] can be useful to detect even very low levels of mutation. Boisselier et al. firstly reported the possibility to detect IDH1^{R132H} mutation amplifying small-size tumor DNA from plasma of glioma patients [132]. In a recent work, Martinez-Ricarte et al. were able to define the mutational status of different genes, including IDH1/2, analyzing the CSF cell-free circulating tumor DNA. However, the technique lacked sensitivity in detecting ctDNA in three out of the five IDH mutant low grade gliomas in this study [133]. Another source of circulating tumor nucleic acids are extracellular vesicles (EV). Mutant IDH1^{R132H} mRNA has been identified in CSF-, but not serum-, -derived EV of patients with mutated glioma [134].

Blood-brain barrier disruption (as reflected by contrast enhancement on MRI) and/or necrosis are associated with a higher amount of free circulating tumor DNA and higher probability to detect the mutation [132–134]. This appear as a limit as the majority of IDH mutant gliomas are non-enhancing gliomas, making the detection of tumor nucleic acids poorly sensitive in these entities.

1.5 IDH mutation as a theranostic marker

1.5.1 IDH inhibition

As IDH mutation is an early, truncal and stable alteration leading to the production of a specific oncometabolite, the inhibition of the neomorphic enzymatic activity soon appeared as a logic therapeutic approach. Since the first report of an *in vitro* effective IDH1^{R132H} inhibitor [104], several other inhibitors targeting also non-R132H IDH1 and IDH2 mutations have been developed [135–138].

Preclinical studies showed that IDH mutant inhibition cause a detectable decrease in D2HG levels and the induction of differentiation in IDH mutant glioma cells [104,135,136]. Kopinja et al. [136] . reported a decrease in 5-methylcytosine levels in treated tumor sections compared to controls. However, no detectable effects on global DNA methylation levels were seen when directly analyzed [104,135]. A reduction of repressive histone trimethylation markers was identified in cells treated with higher doses of the inhibitor [104]. Murine models showed promising results in terms of tumor growth inhibition and increased survival [104,135,136,139]. However, IDH mutant inhibition did not result in mutant cells growth blockade in other studies, despite effective D2HG levels reduction, suggesting that in some lines tumor growth is independent from IDH mutant function [136,140]. As exposed above, IDH mutation at the late stages of glioma evolution is no longer driver nor necessary for tumor maintenance. Furthermore, number of epigenetic changes induced by D2HG are irreversible and remain fixed, even on withdraw of IDH mutation and D2HG [141]. Several phase I studies in humans have been developed in the last years. Final results of phase I trial of ivosidenib (AG-120) in AML have been recently published showing a favorable safety profile and encouraging response rate [142]. First data from phase I trials of AG-120 and AG-881 in gliomas has been presented in abstract form, showing a good safety profile and reporting prolonged disease control in a number of patients, mostly with non-contrast-enhancing tumors [143,144]. In line with the experimental data exposed above, this result may suggest that the inhibition of the neomorphic should be proposed at the early stage of the disease.

On the other hand, we may expect that the inhibition of neomorphic enzyme will trigger immune response. Indeed immunity to syngeneic *IDH1* mutant tumors induced by *IDH1*-specific peptide vaccination or checkpoint inhibition was improved by pharmacological blockade of the neomorphic enzymatic function of mutant *IDH1* [58].

1.5.2 Demethylating agents

Since IDH mutation exerts most of its effect through DNA hypermethylation, the utility of demethylating drugs in gliomas has been explored. Decitabine and 5-azacytidine are two FDA-approved DNA methyltransferase (DNMT) inhibitors; in preclinical studies they both showed to reduce DNA hypermethylation and induce differentiation of glioma cells [145,146]. A phase I study with 5-azacytidine in hematological and solid tumors (including gliomas) is currently ongoing (clinicaltrials.gov identifier: NCT02223052).

1.5.3 IDH vaccination

The IDH mutation creates a tumor-specific, uniformly expressed neoantigen, theoretically suitable for targeted immunization. Murine models confirmed that vaccination with a peptide containing the mutated IDH1^{R132H} epitope can elicit a mutation-specific acquired immune response [147,148]. The feasibility and effectiveness of IDH vaccines are currently explored in the human setting. The German NOA-16 [149] phase I trial of IDH1^{R132H} vaccination in IDH mutant grade III-IV gliomas (clinicaltrials.gov identifier: NCT02454634) completed the data accrual and first results have been presented in abstract form. The induction of acquired humoral and/or T cell responses has been detected in most of the patients vaccinated [150]. Final results are expected for the end of the current year. The RESIST phase I trial of PEPIDH1M vaccine [151] in recurrent IDH mutant grade II gliomas (clinicaltrials.gov identifier: NCT02193347) is currently recruiting. In order to potentiate the effect of vaccination, the AMPLIFY-NEOVAC (EudraCT number:2017-000587-15) evaluate the combination of IDH1 mutation-specific peptide vaccination with PD-L1 checkpoint inhibition.

1.5.4 Synthetic lethality

Sulkowski et al. [80]. recently reported that IDH mutant cells harbor an intrinsic double-strand DNA break repair defect as a result of a deficit in homologous recombination (HR). D2HG induces HR impairment by competitive inhibition of αKG-dependent dioxygenases KDM4A/B. Pharmacological inhibition of the poly(ADP-ribose) polymerase (PARP), an enzyme implicated in HR pathways, induced a state of marked synthetic lethality in IDH mutant cells. IDH mutant cells sensitivity to PARP inhibition has been consistently shown in a different work [152]. Olaparib (an FDA-approved PARP inhibitor) administration in xenograft models caused a significant tumor growth delay [80]. Furthermore, olaparib enhanced temozolomide sensitivity of IDH mutant cells. Based on these results, a phase II study of olaparib in recurrent IDH mutant gliomas (OLAGLI) has been planned (clinicaltrials.gov identifier: NCT03561870).

There is recent evidence that IDH mutant cells have reduced expression of NAPRT1, a rate-limiting enzyme in NAD+-salvage pathways, via promoter hypermethylation. Tateishi et al. [140] demonstrated that blocking a different NAD+-salvage pathway via the inhibition of the NAMPT enzyme induces a strong reduction of intracellular NAD+ levels, impairment of tricarboxylic acid cycle and activation of metabolic-sensing pathway of autophagy, finally resulting in cell death. Testing on murine xenograft models confirmed a strong, IDH-dependent inhibition of tumor growth. NAMPT inhibitors have been reported as temozolomide sensitizers in glioblastoma cells [153], but to our knowledge no study has been performed to evaluate their utility in patients with IDH mutant gliomas.

Furthermore, IDH mutated tumors appear to be exquisitely sensitive to inhibitors of Bcl-xL, an anti-apoptotic gene of the Bcl-2 family. Tumors usually upregulate Mcl-1 gene expression as an escape pathway from Bcl-xL inhibition; in IDH mutated tumors, D2HG downregulates Mcl-1, inducing a state of

synthetic lethality. Murine xenograft models confirmed a survival benefit from ABT263, a specific Bcl-xL inhibitor [154].

Lastly, D2HG production in IDH mutant gliomas is dependent on glutaminase enzymatic activity. Glutaminase is an enzyme involved in α-KG production pathway, hydrolyzing glutamine to glutamate. *In vitro* inhibition of glutaminase with a specific small molecule inhibitor selectively impaired tumor growth of IDH mutant, but not wildtype, tumor models. Intriguingly, D2HG levels remained stable, suggesting a different target of activity [155]. Of note, glioma cells may bypass glutaminase blocking via direct uptake of glutamate from the brain microenvironment [156,157]. A phase 1b trial of a glutaminase inhibitor (CB-839) in association with temozolomide and radiotherapy has been planned (clinicaltrials.gov identifier: NCT03528642).

2 Conclusion

IDH status is the major classifier of diffuse gliomas and is now included in the WHO 2016 glioma classification. It is a major diagnostic, prognostic, predictive marker in gliomas–and possibly theranostic in the next future. It also represents an opportunity for non-invasive diagnosis based on liquid biopsy (D2HG measurement and/or IDH mutation detection) or MR spectroscopy. Because IDH mutation is the earliest and the most stable genetic alteration, and because it inhibits host immune response, IDH mutant tumors represents an ideal target for specific inhibitors and vaccination.

3 Expert commentary

The knowledge of the IDH status is mandatory for the management of glioma patients, as it impacts both patients prognosis and treatment choices. Widely available techniques easily performed in routine are nowadays available for IDH status determination on tissue samples.

An integrated diagnosis combining the IDH1^{R132H} immunostaining and standard Sanger sequencing, when needed, is informative and sufficient in the vast majority of the cases. All results should always be interpreted taking into account sample provenience (tumor core versus invasion margins) and histological appearance in order to suspect possibly false negative results.

Several techniques with faster processing and higher analytical sensitivity compared to standard Sanger sequencing have been defined in the last years, but no one emerged as the new standard. They are usually chosen based on single laboratory expertise.

IDH mutation represents an opportunity for non-invasive diagnosis based on liquid biopsy (D2HG measurement and IDH mutation detection in body fluids) or MR spectroscopy. In contrast to other IDH mutant neoplasms, the liquid biopsy in IDH mutant gliomas still lacks sensitivity, probably due to the relatively small tumor mass, and also to the presence of an intact blood-brain barrier in low grade, non-enhancing gliomas which limits the free DNA -but not the D2HG- release. Overall mutant DNA or D2HG dosage appear more reliable and easier to detect in the CSF compared to the serum/plasma. In parallel, several centers throughout the world have developed a reliable MR spectroscopy method to detect D2HG. However, none of these techniques is currently use in routine nowadays.

It still remains unclear why results with mutant IDH inhibitors have been less promising in gliomas than for myeloid leukemia. This is probably not due to the pharmacokinetics because we have several cases of patients non responder to IDH mutant inhibition in which MR spectroscopy clearly showed a decrease in D2HG levels (unpublished data). Alternatively, the reversibility of IDH mutant-induced epigenetic changes may also be dependent on tissue type, glial cells being possibly less prone to reverse epigenetic changes after D2HG withdrawal [141]. Importantly, as explained above, the acquisition of tertiary driver alterations over time, while IDH mutation becomes a passenger alteration (and may even be eliminated), suggest that maximal benefit for mutant IDH-targeted therapy occur when it is initiated early after initial diagnosis.

Finally, IDH mutations offer a unique opportunity for personalized treatments: these should be based on the deep knowledge of the pleiotropic effects of IDH mutation in tumor cell and microenvironment, and its role along the whole natural history of gliomas. There is therefore a need to better understand the consequences of IDH mutation: in first, the effects mediated by D2HG (on epigenetic and gene expression, particularly modifying the 3D folding of the chromatin but not only, because D2HG competitively inhibits a great variety of cellular enzymes), but also by different mechanisms (metabolic imbalances as the decrease of NADPH/NADP ratio). It also includes the paracrine effects of D2HG on the microenvironment (lymphocytes, macrophages, neurons). As a consequence, IDH inhibitors should be proposed early (see above). Secondly, IDH status uncover metabolic vulnerabilities that can be taken advantage to induce a specific synthetic lethal state, such as with PARP inhibition, NAD+ depletion, glutaminase inhibition. Finally IDH mutant gliomas may be excellent candidates for immunotherapeutic approaches: IDH mutant inhibitors showed to be able to disrupt the immunosuppressive paracrine effects of D2HG and can be combined with anti IDH mutant vaccination and/or checkpoint inhibitor therapies. Combined approaches appear thus promising and are likely to be one of the explored paths in the next years.

4 Five-year view

The last years have seen the affirmation of IDH mutation as the main prognostic and predictive marker in diffuse gliomas. The field is moving fast, and the next years will bring a better knowledge on the pleiotropic mechanism of the neomorphic IDH enzyme, exploring the effect of IDH mutation on epigenetic, gene expression and cellular metabolism, and the effect of D2HG on microenvironment and immune response. In addition, the recent identification of loci associated with the risk of developing specifically IDH mutant gliomas will also help to dissect the mechanism underlying IDH mutant gliomas, as compared to IDH wildtype [158,159].

There is a need to improve sensitivity and specificity of non-invasive diagnosis of IDH mutant gliomas. The most

reliable technique at the moment is the D2HG-detecting MR spectroscopy, which is likely to enter into clinical practice for preoperative evaluation in most centers; its utility in longitudinal follow up will be further defined.

The most important progresses are expected from new therapeutic approaches directed to the inhibition of or vaccination against the neomorphic enzyme, or taking advantage of synthetic lethality.

Based on positive safety results obtained from phase I studies, several IDH inhibitors are going to be explored in phase II trials. Further from their use in monotherapy, they have promising synergistic effect with immunotherapy.

A deeper comprehension of mutated cells metabolism and their intrinsic vulnerabilities induced by D2HG accumulation will lead to the development of new synthetic lethal drugs, while clinical trials will explore the feasibility and effectiveness of established ones.

Key issues

- Mutation of IDH1/2 genes is the first known genetic alteration in the vast majority of grade II and III diffuse gliomas, and lead to gliomagenesis mostly via the production of the oncometabolite D2HG and subsequent epigenetic remodeling.
- IDH mutation is the main molecular prognostic and predictive marker in diffuse gliomas.
- IDH mutation is a major classifier and is integrated in the 2016 revision of WHO classification of central nervous system tumors. Diffuse gliomas are classified into three main groups: IDH mutant – which in turn are separated into chromosomes 1p/19q codeleted and not-codeleted – and IDH wildtype gliomas.
- IDH status determination is easy to assess in routine and includes the immunostaining for the most common *IDH1*^{R132H}mutationanddirect sequencing of codons IDH1 R132 and IDH2 R172 to detect the less frequent mutations.
- IDH mutation represents a unique opportunity for non-invasive diagnosis, based on mutation detection from free circulating DNA and/or D2HG detection by dosage (in the CSF) or by *in vivo* MR spectroscopy.
- IDH is a theranostic marker: innovative therapies are currently developed in clinical trials, directed toward specific inhibition of IDH mutant enzymes, specific vaccination against the mutant enzyme, or uncovering synthetic lethality, for example wit PARP inhibitors.

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