Carcinogenesis, 2019, Vol. 40, No. 11, 1299–1307

doi:10.1093/carcin/bgz134
Advance Access Publication September 3, 2019
Review

REVIEW

Friend or foe—*IDH1* mutations in glioma 10 years on

L. Eric Huang[1,2,*]

[1]Department of Neurosurgery, Clinical Neurosciences Center and [2]Department of Oncological Science, Huntsman Cancer Institute, University of Utah, 175 North Medical Drive East, Salt Lake City, UT 84132, USA

*To whom correspondence should be addressed. Tel: +1 801 585 3221; Fax: +1 801 581 7005; Email: eric.huang@hsc.utah.edu

Abstract

The identification of recurrent point mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene, albeit in only a small percentage of glioblastomas a decade ago, has transformed our understanding of glioma biology, genomics and metabolism. More than 1000 scientific papers have been published since, propelling bench-to-bedside investigations that have led to drug development and clinical trials. The rapid biomedical advancement has been driven primarily by the realization of a neomorphic activity of *IDH1* mutation that produces high levels of D-2-hydroxyglutarate, a metabolite believed to promote glioma initiation and progression through epigenetic and metabolic reprogramming. Thus, novel inhibitors of mutant IDH1 have been developed for therapeutic targeting. However, numerous clinical and experimental findings are at odds with this simple concept. By taking into consideration a large body of findings in the literature, this article analyzes how different approaches have led to opposing conclusions and proffers a counterintuitive hypothesis that *IDH1* mutation is intrinsically tumor suppressive in glioma but functionally undermined by the glutamate-rich cerebral environment, inactivation of tumor-suppressor genes and *IDH1* copy-number alterations. This theory also provides an explanation for some of the most perplexing observations, including the scarcity of proper model systems and the prevalence of *IDH1* mutation in glioma.

Like almost all human cancers, malignant gliomas in the central nervous system are, in essence, a genetic disease (1,2), originating from astrocyte-like neural stem cells in the subventricular zone that harbor oncogenic mutations (3). A tremendous amount of effort has been made toward uncovering the genetic alterations underlying gliomagenesis (4,5), especially in the most malignant form—glioblastoma of World Health Organization (WHO) grade IV [see refs (6,7) for glioma classification and patient demographics]. Somatic mutations target oncogenes and tumor-suppressor genes, whereas 'driver' mutations are positively selected for their ability to confer growth advantage via the acquisition of oncogenic activity concomitant with the inhibition of tumor-suppressive activity, the rest are 'passenger' mutations lacking defined roles (2).

Accordingly, numerous types of genetic alteration have been identified in glioblastoma (4,5,8). Notably, loss of heterozygosity in chromosome 10q, oncogenic amplification of *EGFR* (epidermal growth factor receptor) and homozygous deletion or mutation of the tumor-suppressor genes *CDKN2A* (cyclin-dependent kinase inhibitor 2A) and *PTEN* (phosphatase and tensin homolog) are found in primary (*de novo*) glioblastomas, whereas mutations in the tumor-suppressive *TP53* are commonly detected in secondary (progressive) glioblastomas derived from diffuse astrocytomas of WHO grade II and grade III (referred to collectively as lower-grade glioma hereafter) (4,6). Overall, various genetic alterations converge onto three core pathways in glioblastoma: the oncogenic receptor tyrosine kinase/RAS/phosphatidylinositide 3-kinase pathway and the two tumor-suppressive TP53 and RB pathways (5). Although the frequencies of genetic alterations among the core pathways vary between 78 and 88%, recurrent changes in a single gene occur at much lower frequencies (Table 1).

IDH1 mutations

When recurrent heterozygous mutations in the cytosolic isocitrate dehydrogenase 1 (*IDH1*) gene were first reported in glioblastoma, the mutation frequency was only 12% (9); however, it was remarkable that (i) all *IDH1* mutations targeted the same codon Arg132, which is evolutionarily conserved and

Received: April 15, 2019; **Revised:** July 2, 2019; **Accepted:** July 25, 2019

Abbreviations

5hmC	5-hydroxymethylcytosine
D-2HG	(D)-2-hydroxyglutarate
HIF	hypoxia-inducible factor
IDH1	isocitrate dehydrogenase 1
PDGF	platelet-derived growth factor

Table 1. Recurrent genetic alterations in glioblastomas

Genetic alteration	Frequency (5)
CDKN2A homozygous deletion or mutation	52%
CDKN2B homozygous deletion	47%
EGFR mutation or amplification	45%
PTEN mutation or homozygous deletion	36%
TP53 mutation or homozygous deletion	35%

functionally required for the interaction with the substrate isocitrate; (ii) *IDH1* mutations were found preferentially in younger patients, nearly all with secondary glioblastomas; and (iii) patients with *IDH1* mutation had median overall survival three times longer than those without. Subsequent studies not only confirmed the high frequency of $IDH1^{R132}$ mutations in secondary glioblastomas (>85%) but also revealed the prevalence in lower-grade gliomas (>68%) (10,11) (Table 2). Among lower-grade glioma with *IDH1* mutations, the substitution of Arg132 with histidine—$IDH1^{R132H}$—occurred 92% of the time, whereas $IDH1^{R132C}$, $IDH1^{R132G}$, $IDH1^{R132S}$ and $IDH1^{R132L}$ were at much lower frequencies (12). Lower-grade gliomas without *IDH1* mutations often acquired mutations in the mitochondrial *IDH2* gene at Arg172 (11), which is functionally analogous to IDH1 Arg132 (13). Overall, 95% of these mutations were identified in *IDH1* and only 5% in *IDH2* (11,14) (Table 2). Thus, *IDH1* mutations will be discussed hereafter with $IDH1^{R132H}$ as the archetype.

It is noteworthy that $IDH1^{R132H}$ is rare in primary glioblastomas (<5%) and non-existent in other types of brain tumors (10,11). Furthermore, *IDH1* and *IDH2* mutations are rare or non-existent in cancer types outside of the central nervous system (11,15,16), with the exception of cartilaginous tumors, leukemia and lymphoma, and intrahepatic cholangiocarcinomas (17,18) (Table 2). Importantly, $IDH1^{R132H}$ confers a distinctive survival advantage in glioma patients; large cohort studies confirmed a 2-fold increase of median overall survival in glioblastoma patients and a more than threefold increase in lower-grade glioma patients compared with their respective controls (11,19). Furthermore, multivariate analysis confirmed that $IDH1^{R132H}$ was an independent favorable prognostic marker in gliomas (20). Moreover, maximal resection or combined temozolomide with radiotherapy confers survival benefit specifically on patients with $IDH1^{R132H}$ astrocytomas without 1p/19q codeletion but not those with 1p/19q codeletion or *IDH1* wild-type gliomas (21).

IDH1 mutation is neomorphic

The vast majority of somatic mutations in cancer genes are dominantly acting, i.e., a single-allele mutation is sufficient to be oncogenic, whereas inactivation of tumor-suppressor genes often requires mutation of both alleles (2). The IDH1 enzyme catalyzes the conversion of isocitrate and $NADP^+$ to 2-oxoglutarate and NADPH (Figure 1a). Given the heterozygous nature of $IDH1^{R132H}$, a feed-forward mechanism was initially speculated by which the mutation abrogates the negative feedback to increase NADPH production (22) because NADPH is a reducing equivalent critical for biosynthesis and redox homeostasis (23). Consistent with its role in glioma growth, *IDH1* was found to be overexpressed in glioblastoma and essential to tumor growth (24). However, the fact that $IDH1^{R132H}$ is associated with decreased NADPH production (11,25–29) apparently argues against this notion.

Gene mutations in the mitochondrial succinate dehydrogenases and fumarate hydratase of the citric acid cycle have been linked to paraganglioma and leiomyosarcoma, respectively (30). These loss-of-function mutations result in respective accumulation of succinate and fumarate, which inhibits the 2-oxoglutarate-dependent HIF (hypoxia-inducible factor) prolyl 4-hydroxylases (EGLN) that negatively regulate HIF-1—a transcription factor implicated in tumor angiogenesis and glycolysis (31) (Figure 1b). Thus, the notion that $IDH1^{R132H}$ is a loss-of-function mutation was proposed based on the dominant $IDH1^{R132H}$ inhibition of wild-type IDH1 catalytic activity through heterodimerization, thereby reducing the production of 2-oxoglutarate and in turn enhancing HIF-1 activity (32). Subsequent studies confirmed the heterodimeric interaction but questioned the dominant-negative effect of $IDH1^{R132H}$ and the stimulation of HIF-1 signaling in gliomas (33,34).

Lastly, a landmark discovery established that $IDH1^{R132H}$ is, in fact, neomorphic, resulting in a dominant gain-of-function that catalyzes the NADPH-dependent reduction of 2-oxoglutarate to (D)-2-hydroxyglutarate [D-2HG or R-2HG] but not the enantiomer L-2HG or *S*-2HG (35) (Figure 1a). Furthermore, $IDH1^{R132H}$ gliomas generally had up to 100-fold higher levels of D-2HG than those without the mutation. Similarly, high D-2HG levels were also detected in the tumor tissues and sera of leukemia patients with *IDH1* and *IDH2* mutations (13). Therefore, $IDH1^{R132H}$ acquires a neomorphic enzymatic activity to produce D-2HG.

D-2HG induces epigenetic reprogramming

D-2HG is a competitive inhibitor of multiple 2-oxoglutarate-dependent dioxygenases, including EGLN, histone demethylases and the TET (ten-eleven translocation) family of 5-methylcytosine hydroxylases (36) (Figure 1a). In particular, histone demethylases are 200-fold more sensitive to D-2HG than EGLN, suggesting the involvement of $IDH1^{R132H}$ in chromatin remodeling (37). Indeed, exogenous $IDH1^{R132H}$ induced impairment of histone demethylation apparently preceding DNA hypermethylation (38). The greater accumulation of a repressive mark—trimethylation of lysine 9 in histone 3 (H3K9me3)—was particularly noticeable in preceding the changes in DNA methylation, resulting from specific inhibition of the demethylase KDM4C (Figure 1a). In endogenous $IDH1^{R132H}$-heterozygous cells, global and gene-specific H3K9me3 correlated with locus-specific DNA hypermethylation of downregulated genes (39). Furthermore, AGI-5198—a potent $IDH1^{R132H}$ inhibitor—induced demethylation of histone H3K9me3 and expression of genes associated with differentiation in endogenous $IDH1^{R132H}$ glioma cells (40). Similarly, AGI-6780—a potent inhibitor of $IDH2^{R140Q}$—induced differentiation of leukemia cells (41). Therefore, D-2HG induces histone methylation and blocks cell differentiation.

Likewise, exogenous $IDH1^{R132H}$ was sufficient to induce DNA hypermethylation in immortalized primary human astrocytes in a pattern resembling the glioma-CpG island methylator phenotype (G-CIMP) (42) (Figure 1a)—an associated feature of gliomas harboring *IDH1* mutations (43). Genetically engineered heterozygous $IDH1^{R132H}$ in human colon cancer HCT116 cells and SV40-immortalized human astroglial cells also induced genome-wide

Table 2. *IDH1* and *IDH2* point mutations in human cancers

Cancer type	Frequency	Most frequent	*IDH1*:*IDH2*	Outcome
Primary glioblastomas	<5%	$IDH1^{R132H}$	100:0	Beneficial
Secondary glioblastomas	>85%	$IDH1^{R132H}$	100:0	Beneficial
Lower-grade gliomas	>68%	$IDH1^{R132H}$	95:5	Beneficial
Cartilaginous tumors	>56%	$IDH1^{R132C}$	92:8	Insignificant
Angioimmunoblastic T-cell lymphoma	>20%	$IDH2^{R172K}$	0:100	Insignificant
Acute myeloid leukemias	<15%	$IDH1^{R132C}$ and $IDH2^{R140Q}$	53:47	Adverse or insignificant
Intrahepatic cholangiocarcinomas	<12%	$IDH1^{R132C}$	89:11	Beneficial
Other cancer types	<3%	ND	ND	ND

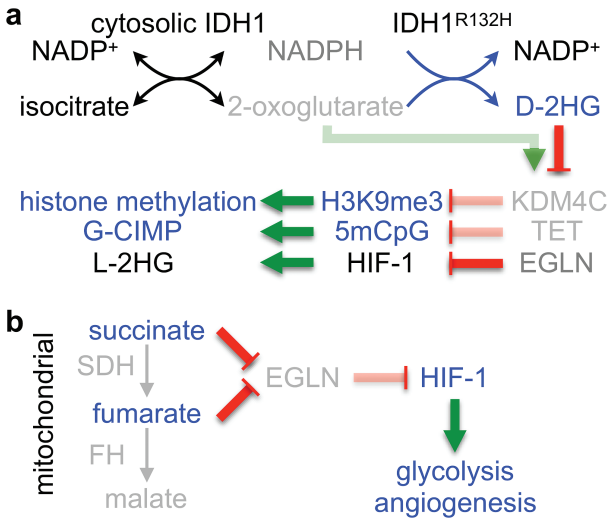

Figure 1. Heterozygous $IDH1^{R132H}$ induces epigenetic and metabolic reprogramming by inhibiting 2-oxoglutarate-dependent dioxygenases in glioma. (**a**) A diagram depicts the $IDH1^{R132H}$ neomorphic activity in the cytosol that catalyzes D-2HG production through the hydroxylation of 2-oxoglutarate from IDH1-mediated oxidation of isocitrate. Consequently, D-2HG acts as an antagonist of 2-oxoglutarate primarily to inhibit histone demethylase KDM4C and 5-methylcytosine hydroxylase TET, resulting in histone methylation (H3K9me3) and CpG methylation (5mCpG). High levels of D-2HG induce HIF-1 signaling by inhibiting HIF prolyl 4-hydroxylase EGLN and subsequently induce L-2HG production. (**b**) Accumulation of succinate and fumarate in the mitochondria, resulting from mutations in the genes encoding succinate dehydrogenase (SDH) and fumarate hydratase (FH), respectively, inhibits HIF prolyl 4-hydroxylase EGLN, thereby stimulating HIF-1 signaling for tumorigenesis. Enhanced events are highlighted in solid color, whereas inhibited events are shaded gray.

alterations in DNA methylation including hypermethylation and, to a lesser extent, hypomethylation of CpG loci (39,44). The TET 5-methylcytosine hydroxylases catalyze the oxidation of 5-methylcytosine into 5-hydroxymethylcytosine (5hmC), thereby maintaining gene promoters in an unmethylated state for gene activation (45). Although exogenous $IDH1^{R132H}$ was shown to inhibit the 5-methylcytosine hydroxylase activity and reduce 5hmC conversion (36,42), the relationship between $IDH1^{R132H}$ and 5hmC in glioma remains controversial (36,46,47). In fact, 5hmC depletion was found in many types of human cancer (46,48) and was associated with poor survival of glioma patients (47,49). Hence, $IDH1^{R132H}$ induces G-CIMP-like epigenetic reprogramming, but its involvement in 5hmC depletion remains less clear in glioma.

Is *IDH1* mutation oncogenic?

$IDH1^{R132H}$ is believed to drive gliomagenesis through the 'oncometabolite' D-2HG to induce epigenetic, metabolic and transcriptional alterations (17,18,22,50). There is abundant, albeit circumstantial, evidence to support this concept. First, $IDH1^{R132H}$ is recurrent and neomorphic, supporting a gain of function in a proto-oncogene. Second, $IDH1^{R132H}$ is believed to be an early genetic event that confers a growth advantage for glioma initiation (19,51). Third, $IDH1^{R132H}$ is genetically preserved in recurrent gliomas (52–54). Fourth, exogenous $IDH1^{R132H}$ is sufficient to induce G-CIMP, block cell differentiation, and initiate oncogenic transformation (34,38,42). Finally, potent IDH1 inhibitors were shown to promote differentiation, inhibit tumor growth and extend animal survival (40,55). The fact that these inhibitors are in clinical trials epitomizes the rapid biomedical advancements from concept to bedside since the seminal discovery of *IDH1* mutations.

Cautionary observations

Although the ongoing clinical trials hold the promise of eventual reduction of morbidity and mortality, the optimism of success has yet to be reconciled with indisputable clinical and experimental observations that seem to suggest caution. First, despite its prevalence in glioma, heterozygous $IDH1^{R132H}$ is scarcely preserved in cell culture or patient-derived xenografts (56–59). As such, exogenous $IDH1^{R132H}$ has almost become the norm of investigation (34,38,40,42,55,60); however, none of the resultant findings have been corroborated in endogenous $IDH1^{R132H}$-heterozygous models (26,29,44,61,62) (Table 3). Second, the notion that $IDH1^{R132H}$ is oncogenic is based on the comparison of exogenous $IDH1^{R132H}$ with wild-type *IDH1*, which seems at odds with the observation that patients with $IDH1^{R132H}$ glioma have better survival than those of *IDH1*-wild type (9,11,19,20). Third, in agreement with the lack of tumor development in heterozygous $Idh1^{R132H}$ mice despite high levels of D-2HG (26,61–63), patients with D-2-hydroxyglutaric aciduria—a rare autosomal recessive disorder characterized by high D-2HG levels in urine, plasma, and cerebrospinal fluid—have no predisposition to tumor development. These patients may have $IDH2^{R140Q}$ and $IDH2^{R140G}$ in the germline and are either asymptomatic or manifest encephalopathy, muscular dystrophy and cardiomyopathy (64,65). Consistently, $IDH2^{R140Q}$ and $IDH2^{R172K}$ transgenic mouse models recapitulated cardiomyopathy and neurodegeneration with no tumor development (66). Of note, L-2-hydroxyglutaric aciduria—another rare autosomal recessive encephalopathy—apparently confers predisposition to brain malignant tumors (67). Finally, studies have indicated that D-2HG is non-essential to glioma progression (29,68,69). Therefore, these observations not only challenge the concept that $IDH1^{R132H}$ is gliomagenic but also call into question the potential efficacy of mutant IDH inhibitors in glioma treatment.

IDH1 mutation—guilty by association

The assessment of $IDH1^{R132H}$ in relation to *TP53* mutation or loss of 1p/19q by comparing the initial and recurrent biopsies from

Table 3. *IDH1*^{R132H}—different approaches, different conclusions

	Tumor suppressive	Oncogenic
IDH1^{R132H}	Endogenous, heterozygous (26,29,44,61,62)	Exogenous (34,42,60,61,70,71)
IDH1^{R132H} expression	Detected in anchorage-dependent culture Suppressed in anchorage-independent culture (29) (P.D.B.Tiburcio *et al.*, unpublished data)	Detected in anchorage-dependent culture (34,42)
Nestin expression	Upregulated in anchorage-independent culture of *IDH1*^{R132H}-hemizygous but not *IDH1*^{R132H}-heterozygous cells (P.D.B.Tiburcio *et al.*, unpublished data)	Upregulated in anchorage-dependent culture transduced with *IDH1*^{R132H} compared with wild-type *IDH1* (38,42)
Anchorage-independent growth	Inhibited by heterozygous *IDH1*^{R132H} Stimulated by loss of *IDH1*^{R132H} heterozygosity, glutamate, and reducing equivalent (29,72) (P.D.B.Tiburcio *et al.*, unpublished data)	Stimulated by exogenous *IDH1*^{R132H} in comparison with wild-type *IDH1* (34,42,70,71)
IDH1^{R132H} effect on tumorigenicity	Non-tumorigenic by itself (26,61,62)	Inducing tumor-like lesions in orthotopic transplantations of astrocytes transduced with *IDH1*^{R132H} compared with wild-type *IDH1* (60,71)
	Decreasing glioma incidence and extending survival in *Trp53*-deficient background (62,73,74) Obliterating gliomagenesis in *Trp53*-intact background when selection against *IDH1*^{R132H} transgene was precluded (72)	Shortening survival in a glioma model of *Atrx*$^{-/-}$; *Cdkn2a*$^{-/-}$; *Pten*$^{-/-}$ background in comparison with wild-type *IDH1* (75)

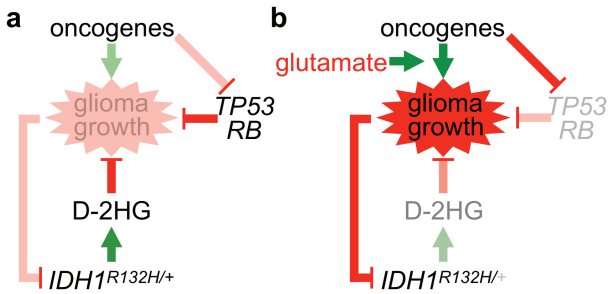

Figure 2. Mechanisms for undermining tumor-suppressive activity of heterozygous *IDH1*^{R132H} during glioma progression. (**a**) In the absence of extracellular glutamate, heterozygous *IDH1*^{R132H}, together with intact tumor-suppressor genes (e.g. *TP53* and *RB*), obliterates oncogenic promotion of gliomagenesis. (**b**) Glutamate in the cerebral cortex negates *IDH1*^{R132H} suppression of gliomagenesis, thereby driving anchorage-independent growth and glioma progression, which is further exacerbated by the inactivation of tumor-suppressor genes and the selection against *IDH1*^{R132H} heterozygosity. The inhibited events are shaded gray.

the same individuals suggested that *IDH1*^{R132H} precedes the other known genetic events and is therefore an early event in the development of lower-grade gliomas (51). Integrated genomic analysis further indicated that the expression of IDH1^{R132H} protein preceded the production of mutant p53 protein and copy-number alterations of *PTEN* and *EGFR* in secondary glioblastoma (76). Further longitudinal analysis showed that *IDH1*^{R132H} was the only event shared in both initial and recurrent gliomas (52,54), although multiregional and temporal samplings revealed additional shared events, including *TERT* promoter mutation and loss of 1p/19q in one case and *TP53* mutation in another (53). Thus, *IDH1*^{R132H} is implicated in glioma initiation and progression, thanks to its tight association.

However, this gliomagenic notion is at odds with the lack of glioma development in both heterozygous *Idh1*^{R132H} mice (26,61,62) and D-2-hydroxyglutaric aciduria patients (64). Although genetic events including inactivation of *TP53* and/or activation of oncogenic signaling, e.g., platelet-derived growth factor (PDGF), are sufficient to induce glioma, *IDH1*^{R132H} has been shown to inhibit glioma penetrance and extend animal survival in comparison with wild-type *IDH1* (62,72–74). Furthermore, the importance of *IDH1*^{R132H} heterozygosity cannot be overemphasized because *IDH1*^{R132H} requires a wild-type allele to produce D-2HG, whereas loss of *IDH1*^{R132H} heterozygosity essentially eliminates D-2HG production (29,77,78). Likewise, genetic alterations at the *IDH1* locus, including *IDH1*^{R132H} amplification, engendered allelic imbalance between *IDH1* and *IDH1*^{R132H}, thereby diminishing D-2HG production in recurrent gliomas (69). Therefore, loss of *IDH1*^{R132H} heterozygosity and *IDH1* allelic imbalance result in non-functional *IDH1*^{R132H} in association with glioma recurrence and progression (Figure 2).

Does *IDH1* mutation-induced epigenetic reprogramming drive gliomagenesis?

DNA hypermethylation has been shown to compromise binding of the methylation-sensitive insulator protein CTCF (CCCTC-binding factor) in IDH-mutant gliomas, resulting in loss of insulation of a neighboring enhancer and aberrant upregulation of the receptor tyrosine kinase gene *PDGFRA* (79). A modest increase of *PDGFRA* transcripts was also observed in IDH-mutant glioma when compared with IDH-wild-type glioma (80). Furthermore, 5hmC enrichment in gene body regions correlated significantly with the upregulation of genes, such as *LGR5* (leucine-rich repeat-containing G-protein-coupled receptor 5), in IDH-mutant glioma (81). It remains unclear, however, whether these epigenetic targets are essential to IDH-mutant gliomagenesis.

In contrast, IDH-mutant gliomas with high levels of DNA methylation had more favorable clinical outcomes than those with low levels (82). A high- to low-level shift in DNA methylation has been identified during glioma recurrence, giving rise to an IDH-wild-type-like glioblastoma phenotype (83). Thus, the loss of DNA methylation is associated with glioma progression. Likewise, silencing of epigenetic targets, such as *LDHA* (lactate dehydrogenase A), *RBP1* (retinol-binding protein), *MIR148A* (microRNA 148a) and *F3* (coagulation factor III, tissue factor), was associated with reduced malignant growth and favorable outcome in IDH-mutant gliomas (59,84–86). Thus, there is insufficient evidence that *IDH1*^{R132H}-induced epigenetic reprogramming drives gliomagenesis.

Does *IDH1* mutation increase cell proliferation?

Exogenous *IDH1*^{R132H} was first shown to stimulate proliferation of late-passaged human astrocytes with inactivated TP53 and RB signaling (87) in reference to wild-type *IDH1* (34). The increased proliferation involved D-2HG stimulation, rather than inhibition (Figure 1a), of EGLN activity and in turn downregulation of HIF signaling as part of cell transformation (34). However, the effect of *IDH1*^{R132H} on hypoxic signaling seems debatable; whereas reduced HIF signaling was observed in IDH-mutant gliomas (59,88,89), increased HIF-1α abundance and target gene expression were also reported in IDH-mutant gliomas and mouse brain-specific *Idh1*^{R132H} knock-in cells (26,32).

With respect to cell proliferation, a large body of evidence indicates that *IDH1*^{R132H} inhibits glial cell proliferation (72,90–93). Mechanistically, D-2HG inhibits ATP synthase, resulting in decreased mTOR (mammalian target of rapamycin) signaling and cell growth (94). Likewise, D-2HG-mediated ATP depletion activates AMPK (5′ AMP-activated protein kinase), thereby inhibiting protein synthesis and mTOR signaling in glioma cells (95). Furthermore, D-2HG promotes cell-cycle arrest by inhibiting the FTO (fat mass and obesity-associated) demethylase activity, thereby increasing N^6-methyladenosine modification of *MYC/CEBPA* (CCAAT/enhancer binding protein alpha) transcripts for destabilization and, in turn, decreasing proliferative signaling (93). Moreover, engineered heterozygous *IDH1*^{R132H} significantly inhibited glial cell proliferation by targeting YAP (Yes-associated protein) and Notch pathways (44). Collectively, these studies provide strong evidence that *IDH1*^{R132H} targets various signaling pathways to inhibit glial cell proliferation at least *in vitro*.

Antagonism between heterozygous *IDH1* mutation and anchorage-independent growth

The cellular ability to proliferate in anchorage-independent growth is consistently associated with tumorigenicity (96). Accordingly, exogenous *IDH1*^{R132H} has been shown to increase anchorage-independent growth of immortalized human astrocytes compared with wild-type control (34,42,60,70,71) (Table 3). Likewise, exogenous *IDH1*^{R132H} resulted in spheroid growth of MYC-immortalized human neural progenitor cells (61). Additional evidence includes the upregulation of neural stem-cell marker genes including *NES* (nestin) in *IDH1*^{R132H}-transduced, late-passaged astrocytes (38,42), albeit under anchorage-dependent conditions. Apparently, these findings support the notion that *IDH1*^{R132H} is gliomagenic; it is unclear, however, whether the exogenous *IDH1*^{R132H} remained expressed in the transduced cells during anchorage-independent growth (see below).

It is striking, however, that none of the abovementioned findings are reproduced in *IDH1*^{R132H}-heterozygous cells; in fact, heterozygous *Idh1*^{R132H} apparently prevented spheroid growth of neural progenitor cells (61). In keeping with this, heterozygous *IDH1*^{R132H} suppressed anchorage-independent growth of glioma cells whereas hemizygous *IDH1*^{R132H} (deficient in wild-type *IDH1* allele) lost the suppression (29) (Table 3). Conversely, anchorage-independent but not anchorage-dependent conditions selected against *IDH1*^{R132H} heterozygosity via a non-genetic mechanism (29) (Figure 2). This antagonism between *IDH1*^{R132H} heterozygosity and anchorage-independent growth not only underscores the functional importance of *IDH1*^{R132H} heterozygosity in glioma biology but also provides an explanation for the frequent loss of either wild-type or mutant *IDH1* allele in patient-derived xenograft, *ex vivo* spheroid culture, and glioma recurrence (57,59,69,77,97). Furthermore, *NES* was strikingly upregulated in anchorage-independent culture of *IDH1*^{R132H}-hemizygous cells compared with *IDH1*^{R132H}-heterozygous cells (P.D.B.Tiburcio *et al.*, unpublished data). Consistently, *NES* expression was significantly higher in IDH-wild-type gliomas than in IDH-mutant gliomas (P.D.B.Tiburcio *et al.*, unpublished data), in agreement with the observation that nestin is an adverse predicator of lower-grade gliomas (98). Therefore, heterozygous *IDH1*^{R132H} is tumor suppressive by inhibiting *NES* expression and anchorage-independent growth. Conversely, anchorage-independent growth selects against *IDH1*^{R132H} heterozygosity, rendering *IDH1*^{R132H} non-functional.

Inactivation of the TP53 and RB pathways renders *IDH1* mutation non-functional

The antagonism between *IDH1*^{R132H} heterozygosity and anchorage-independent growth was also observed in mouse transplantation models, especially in a genetic background without engineered inactivation of tumor-suppressor gene(s), giving rise to tumor growth but no expression of exogenous *IDH1*^{R132H} (72). Exogenous *IDH1*^{R132H} expression was noticeably enhanced when the TP53 and/or RB pathways became deficient (71–73). It is noteworthy that homozygous *Cdkn2a* deletion (affecting both TP53 and RB pathways) resulted in strong IDH1^{R132H} staining in gliomas but complete loss of *IDH1*^{R132H}-associated survival advantage (73), which was also observed with the homozygous deletion of *Cdkn2a* and/or *Pten* in glioma (75). Given the functional requirement of *IDH1* mutation for survival advantage, inactivation of the tumor-suppressor genes renders *IDH1*^{R132H} permissible but non-functional (Figure 2b). As such, the inactivation of both TP53 and RB pathways, e.g., in the immortalized human astrocytes (87), might have obscured *IDH1*^{R132H} tumor-suppressive activity (34,42,60,70,71). Moreover, *IDH1*^{R132H}-associated DNA repair defects and genetic instability (99–101) might have also been relevant, especially in those 'late-passaged' cells (34,42,60).

Extracellular glutamate negates the tumor-suppressive effect of *IDH1* mutation

IDH1^{R132H} malignant glioma arises predominantly from the frontal lobe (76), where the hominoid-specific *GLUD2* (glutamate dehydrogenase 2) gene is evolutionarily selected for the degradation of high flux of glutamate neurotransmitter (102,103). Extracellular metabolites have been shown to negate *IDH1*^{R132H} suppression of anchorage-independent growth (29,72) (P.D.B.Tiburcio *et al.*, unpublished data) (Figure 2b). *IDH1*^{R132H} cells apparently maintain redox homeostasis by increasing glutathione synthesis through the trans-sulfuration pathway and the reliance on glutaminolysis (28,104). In comparison with *IDH1*^{R132H}-heterozygous cells, *IDH1*^{R132H}-hemizygous cells exhibited higher NADPH levels and glutathione/oxidized glutathione ratio (29). It has been proposed that IDH-mutant gliomas depend on glutamate and lactate to alleviate metabolic stress (105,106). Consistently, the addition of glutamate reversed *IDH1*^{R132H} inhibition of PDGF-driven proliferation of neural progenitor cells (92). Likewise, the addition of *N*-acetyl cysteine or glutamate markedly increased anchorage-independent growth of *IDH1*^{R132H}-heterozygous glioma cells (29,72) (P.D.B.Tiburcio *et al.*, unpublished data). The overpowering effect of glutamate was

corroborated by the differential effects of *IDH1*^{R132H} on tumorigenesis between the glutamate-rich cerebral cortex and the subcutaneous tissue in *Trp53*$^{+/+}$ mice; exogenous *IDH1*^{R132H} was virtually ineffective in inhibiting PDGF-driven gliomagenesis in the brain but potently effective in blocking subcutaneous tumor growth (72). Therefore, glutamate may be particularly pertinent to *IDH1*^{R132H} prevalence in glioma but not in most other cancer types (10,11,15,16). Together, these studies indicate the importance of extracellular glutamate for *IDH1*^{R132H} glioma growth.

IDH1 mutation does not initiate gliomagenesis by itself

As discussed above, no glioma development has been seen in heterozygous *Idh1*^{R132H} mice (26,61,62) or in patients with D-2-hydroxyglutaric aciduria (64). Of note, these mice exhibited disorganization of the subventricular zone and epigenetic and transcriptional profiles resembling those of IDH-mutant glioma (61,62). In combination with other genetic events, heterozygous *Idh1*^{R132H} reduced the penetrance of *Trp53* deletion-induced glioma by 70% and extended survival compared with wild-type *Idh1* (62). Likewise, glioma models driven by PDGF in combination with *Trp53* knockdown revealed survival benefits of *IDH1*^{R132H} in RCAS/tva mice (73). Finally, *IDH1*^{R132H} obliterated PDGF-induced gliomagenesis in RCAS/tva mice when selection against exogenous *IDH1*^{R132H} was precluded, demonstrating *IDH1*^{R132H} innate ability for tumor suppression (72) (Figure 2a). Thus, *IDH1*^{R132H} neither initiates nor promotes gliomagenesis.

Studies relying on exogenous *IDH1*^{R132H} expression in TP53/RB-deficient human astrocytes have come to a divergent view (Table 3). Exogenous *IDH1*^{R132H} was shown to overcome telomere-induced crisis by activating *TERT* expression, thereby leading to cell transformation and tumor growth (71). Likewise, long-term *IDH1*^{R132H} expression impaired contact inhibition and yielded intracranial lesions with parenchymal infiltration (60). Moreover, *IDH1*^{R132H} in comparison with *IDH1* shortened mouse survival in a combined *Atrx*$^{-/-}$, *Cdkn2a*$^{-/-}$ and *Pten*$^{-/-}$ background of PDGF-driven glioma models (75). The biological relevance of these studies, however, is less clear because mutations in *CDKN2A* and *PTEN* are rare in IDH-mutant gliomas (19). Furthermore, *IDH1*^{R132H}-induced hydrocephalic neuropathy (26,61,62,66), hypoxic induction of oncogenic L-2HG (107,108) and *IDH1*^{R132H}-associated genetic instability (99–101) might have complicated the interpretations of the studies.

Is *IDH1* mutation a therapeutic target in glioma?

The therapeutic potential of *IDH1* and *IDH2* mutations has been explored extensively; different classes of mutant IDH1 and IDH2 inhibitors have been developed for ongoing phase 1 or phase 1/2 clinical trials of mostly hematological diseases and some solid tumors (109,110). The outcome of these inhibitors in glioma-associated survival studies in mouse models, however, seems less promising; the efficacies vary from modest or indifferent to detrimental despite their high potencies in inhibiting D-2HG production and inducing cell differentiation. AGI-5198 was first shown to decrease subcutaneous growth of *IDH1*^{R132H} glioma cells (40), but tumor regression was not reproduced in another study despite near-complete inhibition of 2HG (111). Likewise, an IDH1$^{R132H/C}$-specific inhibitor (IDHi) (112) had near-complete 2HG elimination but showed no survival benefits in orthotopic transplantation of *IDH1*^{R132H} glioblastoma cells (113). In fact, long-term exposure to IDHi prior to orthotopic transplantation significantly shortened survival (113). The brain-penetrant IDH1^{R132H} inhibitors MRK-A and MRK-B prolonged survival in orthotopic transplantation of one *IDH1*^{R132H} glioma cell type but not another (114). Finally, BAY 1436032—an orally available, pan-IDH1^{R132X} inhibitor—significantly but modestly extended survival in intracranial *IDH1*^{R132H}-astrocytoma transplantation (55). Collectively, mutant IDH1 inhibitors may have therapeutic effects on glioma independent of D-2HG depletion. The first-in-human phase I study (NCT03030066) involving 45 patients with recurrent/progressive *IDH1* mutant glioma has shown favorable tolerance and brain distribution with the pan-IDH1^{R132X} inhibitor DS-1001b (115). Of the 38 evaluable patients in this ongoing trial, 17 (45%) achieved stable disease, 3 (8%) partial response, 2 (5%) minor response, and 1 (3%) complete response.

Of note, mutant IDH1 inhibitors have undesired effects that diminish the efficacy of chemotherapy and radiation therapy. AGI-5198 was shown to reduce radiosensitivity through restoration of NADPH levels, thereby protecting *IDH1*-mutant cells against ionizing radiation (27). It also desensitized cisplatin killing of *IDH1*-mutant cells by reducing levels of reactive oxygen species (116) and completely reversed the sensitivity of cancer cells to poly(adenosine 5′-diphosphate-ribose) polymerase inhibitors (101). In light of the association of non-functional *IDH1*^{R132H} with glioma progression and recurrence, as discussed above, additional biochemical, metabolic and immunological targets should be explored [reviewed in ref. (110)] for the development of better treatment. Likewise, investigating the mechanisms that negate *IDH1*^{R132H} suppression of gliomagenesis (Figure 2) may provide novel targets.

Concluding remarks

IDH1^{R132H} heterozygosity and its association with better survival are hallmarks of gliomas compared with those without the mutation. Whether *IDH1*^{R132H} is a 'friend or foe' depends principally on the model system employed. Studies with heterozygous *IDH1*^{R132H} model systems have demonstrated that *IDH1*^{R132H} is neither a driver nor a passenger but beneficial—another category of somatic mutations in cancer (80). The beneficial effect of heterozygous *IDH1*^{R132H} in comparison with *IDH1*-wild-type or loss of *IDH1*^{R132H} heterozygosity is supported by both clinical and experimental findings discussed above. In contrast, the oncogenic *IDH1*^{R132H} theory is predicated exclusively on the findings of exogenous *IDH1*^{R132H} expression where the selection against *IDH1*^{R132H} heterozygosity by anchorage-independent growth has essentially been overlooked. Despite being intrinsically tumor suppressive, *IDH1*^{R132H} activity can be undermined by various intracellular and extracellular factors, notably inactivation of tumor-suppressor genes, *IDH1*^{R132H} allelic imbalance and selection against *IDH1*^{R132H} heterozygosity, and extracellular glutamate and reducing equivalents. The functional, rather than genetic, loss of *IDH1*^{R132H} provides an explanation for the prevalence and preservation of *IDH1*^{R132H} from glioma initiation to progression. It is imperative, therefore, that investigations be directed toward understanding the mechanism by which *IDH1*^{R132H} functionality is negated for better prevention and therapeutic intervention of glioma progression.

Funding

Department of Neurosurgery at University of Utah, Huntsman Cancer Foundation, and grant P30 CA042014 awarded to Huntsman Cancer Institute and to the NC Program at Huntsman Cancer Institute.

Acknowledgements

I apologize to authors whose work was not cited in this article. I wish to thank Daniel W. Fults for helpful comments and Kristin Kraus for editorial assistance.

Conflict of Interest Statement: None declared.

References

1. Vogelstein, B. *et al.* (2004) Cancer genes and the pathways they control. *Nat. Med.*, 10, 789–799.
2. Stratton, M.R. *et al.* (2009) The cancer genome. *Nature*, 458, 719–724.
3. Lee, J.H. *et al.* (2018) Human glioblastoma arises from subventricular zone cells with low-level driver mutations. *Nature*, 560, 243–247.
4. Ohgaki, H. *et al.* (2007) Genetic pathways to primary and secondary glioblastoma. *Am. J. Pathol.*, 170, 1445–1453.
5. Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*, 455, 1061–1068.
6. Ohgaki, H. *et al.* (2013) The definition of primary and secondary glioblastoma. *Clin. Cancer Res.*, 19, 764–772.
7. Louis, D.N. *et al.* (2016) The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol.*, 131, 803–820.
8. Furnari, F.B. *et al.* (2007) Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev.*, 21, 2683–2710.
9. Parsons, D.W. *et al.* (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science*, 321, 1807–1812.
10. Balss, J. *et al.* (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathol.*, 116, 597–602.
11. Yan, H. *et al.* (2009) IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.*, 360, 765–773.
12. Pusch, S. *et al.* (2014) D-2-hydroxyglutarate producing neo-enzymatic activity inversely correlates with frequency of the type of isocitrate dehydrogenase 1 mutations found in glioma. *Acta Neuropathol. Commun.*, 2, 19.
13. Ward, P.S. *et al.* (2010) The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell*, 17, 225–234.
14. Hartmann, C. *et al.* (2009) Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol.*, 118, 469–474.
15. Bleeker, F.E. *et al.* (2009) IDH1 mutations at residue p.R132 ($IDH1^{R132}$) occur frequently in high-grade gliomas but not in other solid tumors. *Hum. Mutat.*, 30, 7–11.
16. Kang, M.R. *et al.* (2009) Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. *Int. J. Cancer*, 125, 353–355.
17. Dang, L. *et al.* (2010) IDH mutations in glioma and acute myeloid leukemia. *Trends Mol. Med.*, 16, 387–397.
18. Cairns, R.A. *et al.* (2013) Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. *Cancer Discov.*, 3, 730–741.
19. Cancer Genome Atlas Research Network *et al.* (2015) Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N. Engl. J. Med*, 372, 2481–2498.
20. Sanson, M. *et al.* (2009) Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. *J. Clin. Oncol.*, 27, 4150–4154.
21. Tateishi, K. *et al.* (2017) IDH1 Mutation and World Health Organization 2016 diagnostic criteria for adult diffuse gliomas: advances in surgical strategy. *Neurosurgery*, 64, 134–138.
22. Thompson, C.B. (2009) Metabolic enzymes as oncogenes or tumor suppressors. *N. Engl. J. Med.*, 360, 813–815.
23. Pavlova, N.N. *et al.* (2016) The emerging hallmarks of cancer metabolism. *Cell Metab.*, 23, 27–47.
24. Calvert, A.E. *et al.* (2017) Cancer-associated IDH1 promotes growth and resistance to targeted therapies in the absence of mutation. *Cell Rep.*, 19, 1858–1873.
25. Bleeker, F.E. *et al.* (2010) The prognostic *IDH1R132* mutation is associated with reduced $NADP^+$-dependent IDH activity in glioblastoma. *Acta Neuropathol.*, 119, 487–494.
26. Sasaki, M. *et al.* (2012) D-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. *Genes Dev.*, 26, 2038–2049.
27. Molenaar, R.J. *et al.* (2015) Radioprotection of IDH1-mutated cancer cells by the IDH1-mutant inhibitor AGI-5198. *Cancer Res.*, 75, 4790–4802.
28. Fack, F. *et al.* (2017) Altered metabolic landscape in IDH-mutant gliomas affects phospholipid, energy, and oxidative stress pathways. *EMBO Mol. Med.*, 9, 1681–1695.
29. Tiburcio, P.D.B. *et al.* (2018) Functional requirement of a wild-type allele for mutant IDH1 to suppress anchorage-independent growth through redox homeostasis. *Acta Neuropathol.*, 135, 285–298.
30. Tiburcio, P.D. *et al.* (2014) Complex role of HIF in cancer: the known, the unknown, and the unexpected. *Hypoxia (Auckl.)*, 2, 59–70.
31. Huang, L.E. *et al.* (2003) Hypoxia-inducible factor and its biomedical relevance. *J. Biol. Chem.*, 278, 19575–19578.
32. Zhao, S. *et al.* (2009) Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1 alpha. *Science*, 324, 261–265.
33. Jin, G. *et al.* (2011) 2-Hydroxyglutarate production, but not dominant negative function, is conferred by glioma-derived NADP-dependent isocitrate dehydrogenase mutations. *PLoS One*, 6, e16812.
34. Koivunen, P. *et al.* (2012) Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature*, 483, 484–488.
35. Dang, L. *et al.* (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*, 462, 739–744.
36. Xu, W. *et al.* (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell*, 19, 17–30.
37. Chowdhury, R. *et al.* (2011) The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep.*, 12, 463–469.
38. Lu, C. *et al.* (2012) IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature*, 483, 474–478.
39. Duncan, C.G. *et al.* (2012) A heterozygous IDH1R132H/WT mutation induces genome-wide alterations in DNA methylation. *Genome Res.*, 22, 2339–2355.
40. Rohle, D. *et al.* (2013) An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science*, 340, 626–630.
41. Wang, F. *et al.* (2013) Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science*, 340, 622–626.
42. Turcan, S. *et al.* (2012) IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature*, 483, 479–483.
43. Noushmehr, H. *et al.* (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell*, 17, 510–522.
44. Wei, S. *et al.* (2018) Heterozygous IDH1 R132H/WT created by "single base editing" inhibits human astroglial cell growth by downregulating YAP. *Oncogene*, 321, 1807.
45. Branco, M.R. *et al.* (2011) Uncovering the role of 5-hydroxymethylcytosine in the epigenome. *Nat. Rev. Genet.*, 13, 7–13.
46. Jin, S.G. *et al.* (2011) 5-Hydroxymethylcytosine is strongly depleted in human cancers but its levels do not correlate with IDH1 mutations. *Cancer Res.*, 71, 7360–7365.
47. Orr, B.A. *et al.* (2012) Decreased 5-hydroxymethylcytosine is associated with neural progenitor phenotype in normal brain and shorter survival in malignant glioma. *PLoS One*, 7, e41036.
48. Lian, C.G. *et al.* (2012) Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. *Cell*, 150, 1135–1146.
49. Johnson, K.C. *et al.* (2016) 5-Hydroxymethylcytosine localizes to enhancer elements and is associated with survival in glioblastoma patients. *Nat. Commun.*, 7, 13177.
50. Losman, J.A. *et al.* (2013) What a difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer. *Genes Dev.*, 27, 836–852.
51. Watanabe, T. *et al.* (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am. J. Pathol.*, 174, 1149–1153.
52. Johnson, B.E. *et al.* (2014) Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science*, 343, 189–193.
53. Suzuki, H. *et al.* (2015) Mutational landscape and clonal architecture in grade II and III gliomas. *Nat. Genet.*, 47, 458–468.
54. Bai, H. *et al.* (2016) Integrated genomic characterization of IDH1-mutant glioma malignant progression. *Nat. Genet.*, 48, 59–66.

55. Pusch, S. *et al.* (2017) Pan-mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma *in vivo*. *Acta Neuropathol.*, 133, 629–644.
56. Piaskowski, S. *et al.* (2011) Glioma cells showing IDH1 mutation cannot be propagated in standard cell culture conditions. *Br. J. Cancer*, 104, 968–970.
57. Borodovsky, A. *et al.* (2013) 5-Azacytidine reduces methylation, promotes differentiation and induces tumor regression in a patient-derived IDH1 mutant glioma xenograft. *Oncotarget*, 4, 1737–1747.
58. Luchman, H.A. *et al.* (2013) Spontaneous loss of heterozygosity leading to homozygous R132H in a patient-derived IDH1 mutant cell line. *Neuro. Oncol.*, 15, 979–980.
59. Chesnelong, C. *et al.* (2014) Lactate dehydrogenase A silencing in IDH mutant gliomas. *Neuro. Oncol.*, 16, 686–695.
60. Turcan, S. *et al.* (2018) Mutant-IDH1-dependent chromatin state reprogramming, reversibility, and persistence. *Nat. Genet.*, 50, 62–72.
61. Bardella, C. *et al.* (2016) Expression of Idh1R132H in the murine subventricular zone stem cell niche recapitulates features of early gliomagenesis. *Cancer Cell*, 30, 578–594.
62. Pirozzi, C.J. *et al.* (2017) Mutant IDH1 disrupts the mouse subventricular zone and alters brain tumor progression. *Mol. Cancer Res.*, 15, 507–520.
63. Sasaki, M. *et al.* (2012) IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. *Nature*, 488, 656–659.
64. Struys, E.A. (2006) D-2-hydroxyglutaric aciduria: unravelling the biochemical pathway and the genetic defect. *J. Inherit. Metab. Dis.*, 29, 21–29.
65. Kranendijk, M. *et al.* (2010) IDH2 mutations in patients with D-2-hydroxyglutaric aciduria. *Science*, 330, 336.
66. Akbay, E.A. *et al.* (2014) D-2-hydroxyglutarate produced by mutant IDH2 causes cardiomyopathy and neurodegeneration in mice. *Genes Dev.*, 28, 479–490.
67. Aghili, M. *et al.* (2009) Hydroxyglutaric aciduria and malignant brain tumor: a case report and literature review. *J. Neurooncol.*, 91, 233–236.
68. Johannessen, T.-C.A. *et al.* (2016) Rapid conversion of mutant IDH1 from driver to passenger in a model of human gliomagenesis. *Mol. Cancer Res.*, 14, 976–983.
69. Mazor, T. *et al.* (2017) Clonal expansion and epigenetic reprogramming following deletion or amplification of mutant IDH1. *Proc. Natl. Acad. Sci. USA*, 114, 10743–10748.
70. Izquierdo-Garcia, J.L. *et al.* (2015) IDH1 mutation induces reprogramming of pyruvate metabolism. *Cancer Res.*, 75, 2999–3009.
71. Ohba, S. *et al.* (2016) Mutant IDH1 expression drives TERT promoter reactivation as part of the cellular transformation process. *Cancer Res.*, 76, 6680–6689.
72. Tiburcio, P.D.B. *et al.* (2018) IDH1R132H is intrinsically tumor-suppressive but functionally attenuated by the glutamate-rich cerebral environment. *Oncotarget*, 9, 35100–35113.
73. Amankulor, N.M. *et al.* (2017) Mutant IDH1 regulates the tumor-associated immune system in gliomas. *Genes Dev.*, 31, 774–786.
74. Núñez, F.J. *et al.* (2019) IDH1-R132H acts as a tumor suppressor in glioma via epigenetic up-regulation of the DNA damage response. *Sci. Transl. Med.*, 11, eaaq1427.
75. Philip, B. *et al.* (2018) Mutant IDH1 promotes glioma formation *in vivo*. *Cell Rep.*, 23, 1553–1564.
76. Lai, A. *et al.* (2011) Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. *J. Clin. Oncol.*, 29, 4482–4490.
77. Jin, G. *et al.* (2013) Disruption of wild-type IDH1 suppresses D-2-hydroxyglutarate production in IDH1-mutated gliomas. *Cancer Res.*, 73, 496–501.
78. Ward, P.S. *et al.* (2013) The potential for isocitrate dehydrogenase mutations to produce 2-hydroxyglutarate depends on allele specificity and subcellular compartmentalization. *J. Biol. Chem.*, 288, 3804–3815.
79. Flavahan, W.A. *et al.* (2016) Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature*, 529, 110–114.
80. Huang, L.E. *et al.* (2017) IGFBP2 expression predicts IDH-mutant glioma patient survival. *Oncotarget*, 8, 191–202.
81. Glowacka, W.K. *et al.* (2018) 5-Hydroxymethylcytosine preferentially targets genes upregulated in isocitrate dehydrogenase 1 mutant high-grade glioma. *Acta Neuropathol.*, 135, 617–634.
82. Ceccarelli, M. *et al.* (2016) Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell*, 164, 550–563.
83. de Souza, C.F. *et al.* (2018) A distinct DNA methylation shift in a subset of glioma CpG Island methylator phenotypes during tumor recurrence. *Cell Rep.*, 23, 637–651.
84. Chou, A.P. *et al.* (2012) Identification of retinol binding protein 1 promoter hypermethylation in isocitrate dehydrogenase 1 and 2 mutant gliomas. *J. Natl. Cancer Inst.*, 104, 1458–1469.
85. Li, S. *et al.* (2014) Tumor-suppressive miR148a is silenced by CpG island hypermethylation in IDH1-mutant gliomas. *Clin. Cancer Res.*, 20, 5808–5822.
86. Unruh, D. *et al.* (2019) Methylation-dependent tissue factor suppression contributes to the reduced malignancy of IDH1-mutant gliomas. *Clin. Cancer Res.*, 25, 747–759.
87. Sonoda, Y. *et al.* (2001) Formation of intracranial tumors by genetically modified human astrocytes defines four pathways critical in the development of human anaplastic astrocytoma. *Cancer Res.*, 61, 4956–4960.
88. Kickingereder, P. *et al.* (2015) IDH mutation status is associated with a distinct hypoxia/angiogenesis transcriptome signature which is non-invasively predictable with rCBV imaging in human glioma. *Sci. Rep.*, 5, 16238.
89. Miroshnikova, Y.A. *et al.* (2016) Tissue mechanics promote IDH1-dependent HIF1α-tenascin C feedback to regulate glioblastoma aggression. *Nat. Cell Biol.*, 18, 1336–1345.
90. Bralten, L.B. *et al.* (2011) IDH1 R132H decreases proliferation of glioma cell lines *in vitro* and *in vivo*. *Ann. Neurol.*, 69, 455–463.
91. Chittaranjan, S. *et al.* (2014) Mutations in CIC and IDH1 cooperatively regulate 2-hydroxyglutarate levels and cell clonogenicity. *Oncotarget*, 5, 7960–7979.
92. Chen, R. *et al.* (2014) Hominoid-specific enzyme GLUD2 promotes growth of IDH1R132H glioma. *Proc. Natl Acad. Sci. USA*, 111, 14217–14222.
93. Su, R. *et al.* (2018) R-2HG exhibits anti-tumor activity by targeting FTO/m6A/MYC/CEBPA signaling. *Cell*, 172, 90–105.e23.
94. Fu, X. *et al.* (2015) 2-Hydroxyglutarate inhibits ATP synthase and mTOR signaling. *Cell Metab.*, 22, 508–515.
95. Karpel-Massler, G. *et al.* (2017) Induction of synthetic lethality in IDH1-mutated gliomas through inhibition of Bcl-xL. *Nat. Commun.*, 8, 1067.
96. Shin, S.I. *et al.* (1975) Tumorigenicity of virus-transformed cells in nude mice is correlated specifically with anchorage independent growth *in vitro*. *Proc. Natl Acad. Sci. USA*, 72, 4435–4439.
97. Balvers, R.K. *et al.* (2013) Serum-free culture success of glial tumors is related to specific molecular profiles and expression of extracellular matrix-associated gene modules. *Neuro. Oncol.*, 15, 1684–1695.
98. Hatanpaa, K.J. *et al.* (2014) High expression of the stem cell marker nestin is an adverse prognostic factor in WHO grade II–III astrocytomas and oligoastrocytomas. *J. Neurooncol.*, 117, 183–189.
99. Cohen, A. *et al.* (2015) DNA copy number analysis of grade II–III and grade IV gliomas reveals differences in molecular ontogeny including chromothripsis associated with IDH mutation status. *Acta Neuropathol. Commun.*, 3, 34.
100. Inoue, S. *et al.* (2016) Mutant IDH1 downregulates ATM and alters DNA repair and sensitivity to DNA damage independent of TET2. *Cancer Cell*, 30, 337–348.
101. Sulkowski, P.L. *et al.* (2017) 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. *Sci. Transl. Med.*, 9, eaal2463.
102. Burki, F. *et al.* (2004) Birth and adaptive evolution of a hominoid gene that supports high neurotransmitter flux. *Nat. Genet.*, 36, 1061–1063.
103. Shashidharan, P. *et al.* (2014) The discovery of human of GLUD2 glutamate dehydrogenase and its implications for cell function in health and disease. *Neurochem. Res.*, 39, 460–470.
104. McBrayer, S.K. *et al.* (2018) Transaminase inhibition by 2-hydroxyglutarate impairs glutamate biosynthesis and redox homeostasis in glioma. *Cell*, 175, 101–116.e25.

105. Khurshed, M. *et al.* (2017) *In silico* gene expression analysis reveals glycolysis and acetate anaplerosis in IDH1 wild-type glioma and lactate and glutamate anaplerosis in IDH1-mutated glioma. *Oncotarget*, 8, 49165–49177.
106. Lenting, K. *et al.* (2019) Isocitrate dehydrogenase 1-mutated human gliomas depend on lactate and glutamate to alleviate metabolic stress. *FASEB J.*, 33, 557–571.
107. Intlekofer, A.M. *et al.* (2015) Hypoxia induces production of L-2-hydroxyglutarate. *Cell Metab.*, 22, 304–311.
108. Oldham, W.M. *et al.* (2015) Hypoxia-mediated increases in L-2-hydroxyglutarate coordinate the metabolic response to reductive stress. *Cell Metab.*, 22, 291–303.
109. Dang, L. *et al.* (2017) Isocitrate dehydrogenase mutation and (R)-2-hydroxyglutarate: from basic discovery to therapeutics development. *Annu. Rev. Biochem.*, 86, 305–331.
110. Waitkus, M.S. *et al.* (2018) Biological role and therapeutic potential of IDH mutations in cancer. *Cancer Cell*, 34, 186–195.
111. Turcan, S. *et al.* (2013) Efficient induction of differentiation and growth inhibition in IDH1 mutant glioma cells by the DNMT inhibitor decitabine. *Oncotarget*, 4, 1729–1736.
112. Deng, G. *et al.* (2015) Selective inhibition of mutant isocitrate dehydrogenase 1 (IDH1) via disruption of a metal binding network by an allosteric small molecule. *J. Biol. Chem.*, 290, 762–774.
113. Tateishi, K. *et al.* (2015) Extreme vulnerability of IDH1 mutant cancers to NAD+ depletion. *Cancer Cell*, 28, 773–784.
114. Kopinja, J. *et al.* (2017) A brain penetrant mutant IDH1 inhibitor provides *in vivo* survival benefit. *Sci. Rep.*, 7, 13853.
115. Natsume, A. *et al.* (2019) Phase I study of a brain penetrant mutant IDH1 inhibitor DS-1001b in patients with recurrent or progressive IDH1 mutant gliomas. *JCO*, 37, 2004–2004.
116. Khurshed, M. *et al.* (2018) IDH1-mutant cancer cells are sensitive to cisplatin and an IDH1-mutant inhibitor counteracts this sensitivity. *FASEB J*, 32, 6344–6352.