From genomics to the clinic: biological and translational insights of mutant *IDH1/2* in glioma

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The characterization of the genomic alterations across all human cancers is changing the way that malignant disease is defined and treated. This paradigm is extending to glioma, where the discovery of recurrent mutations in the *isocitrate dehydrogenase 1 (IDH1)* gene has shed new light on the molecular landscape in glioma and other *IDH*-mutant cancers. The *IDH1* mutations are present in the vast majority of low-grade gliomas and secondary glioblastomas. Rapidly emerging work on the consequences of mutant *IDH1* protein expression suggests that its neomorphic enzymatic activity catalyzing the production of the oncometabolite 2-hydroxyglutarate influences a range of cellular programs that affect the epigenome, transcriptional programs, hypoxia-inducible factor biology, and development. In the brief time since its discovery, knowledge of the *IDH* mutation status has had significant translational implications, and diagnostic tools are being used to monitor its expression and function. The concept of *IDH1*-mutant versus *IDH1*-wild type will become a critical early distinction in diagnostic and treatment algorithms. (<http://thejns.org/doi/abs/10.3171/2012.12.FOCUS12355>)

KEY WORDS • *isocitrate dehydrogenase 1* • *isocitrate dehydrogenase 2* • glioblastoma • low-grade glioma • magnetic resonance spectroscopy

THE comprehensive taxonomic encyclopedia of the genetic alterations underlying all cancers is rapidly becoming widely available.^{61,85,91} To date, this molecular genomic focus has driven an evolving perspective on malignancies as lesions that are defined by (in addition to microscopic histological findings) their signature tumor genome alterations. Indeed, the lingua franca of this genome-based framework is increasingly pervasive, as is exemplified by lesions such as *HER2*-amplified breast cancer,⁸³ *BCR-ABL*-rearranged chronic myeloid leukemia,²³ *EGFR*-mutant⁵⁵ or *EML4-ALK*-rearranged⁴⁸ non-small cell lung cancer, and *BRAF*-mutant melanoma¹⁶ that populate the growing lexicon of molecular oncology. Importantly, the presence of these alterations is clinically

significant because there are specific inhibitors that target their expressed proteins and lead to dramatic clinical response in patients with these cancers.

Several groups have used a similar approach to glioblastoma, the most common intrinsic brain tumor.^{59,65} Extensive characterization of the genomic changes underlying these cancers has set the stage for a reevaluation of their clinical management. A prototypic example has been the recent identification of mutations in the *IDH1* gene,⁶⁵ a stunning and unexpected discovery that has led to new insights into glioma and cancer biology, has fueled new interest in studies of cancer metabolism, and has clear clinical implications.

Today, neurooncologists and neurosurgeons increasingly stratify glial neoplasms into *IDH1*-mutant or *IDH1*-WT disease.^{24,92} Indeed, the divergent clinical characteristics of histopathologically identical primary (*IDH1*-WT) and secondary (*IDH1*-mutant) glioblastomas are lending further support to the notion that these tumors represent different diseases. Herein we review the discovery and incidence of *IDH* mutation in glioma and other cancers, our current understanding of mutant *IDH* biology, and its increasing translational implications.

Abbreviations used in this paper: α -KG = α -ketoglutarate; AML = acute myeloid leukemia; D-2-HG = D-2-hydroxyglutarate; D-2-HGA = D-2-hydroxyglutaric aciduria; G-CIMP = glioma CpG island methylator phenotype; GFAP = glial fibrillary acidic protein; HIF = hypoxia-inducible factor; *IDH1/2* = isocitrate dehydrogenase 1 and 2; LGG = low-grade glioma; MRS = MR spectroscopy; NADPH = nicotinamide adenine dinucleotide phosphate (reduced form); TCA = tricarboxylic acid; WT = wild type.

Mutations of *IDH1* in Cancer

Discovery of Mutant *IDH1*

In 2008, recurrent mutations in *IDH1* were first identified through work by the Vogelstein group analyzing the DNA sequence of the protein-coding portions of the glioblastoma genome (see Parsons et al.⁶⁵). In that study, 23,219 transcripts from 20,661 protein-coding genes in 22 malignant gliomas were analyzed by capillary-based Sanger sequencing. Of the 22 tumors examined, 7 were derived directly from patient tissue, and 15 were derived from xenografts of human tumors. In this initial so-called Discovery set, 5 of 22 tumors carried an identical mutation of the *IDH1* gene that produced the R132H amino acid substitution of the *IDH1* protein. Notably, the ages of the patients who harbored this mutation in the Discovery set were 30, 31, 32 (2 patients), and 42 years, which is considerably younger than the historically associated age at glioblastoma diagnosis (which is more typically in the 6th or 7th decade of life). Targeted sequencing analysis in a validation sample set of 127 additional tumors identified 13 *IDH1* mutations, including 4 (80%) of 5 tumors known to be secondary glioblastomas. Parsons et al.⁶⁵ provided 2 additional important observations that have since been corroborated by other groups. First, the median age of patients with the *IDH1*-mutant tumors was 33 years, whereas in the patient group with *IDH1*-WT tumors, the median age was 53 years. Second, patients with *IDH1*-mutant tumors demonstrated a significantly longer overall survival time compared with patients in the *IDH1*-WT group.

Of the 3 human *IDH* isoforms, *IDH1* is located in the cytoplasm and peroxisomes, whereas *IDH2* and *IDH3* are located within the mitochondria. The *IDH* proteins are known to catalyze the oxidative decarboxylation of isocitrate to α -KG, leading to the production of NADPH in the TCA cycle, a biochemical sequence critical in sugar, lipid, and amino acid metabolism.⁷⁰ Prior to the study of Parsons et al., these proteins had not previously been linked to cancer. Although a mutation in *IDH1* producing the R132C substitution had been identified previously in patients with colon cancer,⁸² the study of glioblastoma was the first to demonstrate that *IDH1* was recurrently mutated in cancer and correlated with important clinical parameters.

In addition to mutations in *IDH1*, additional components of the TCA cycle are mutated in other non-CNS cancers. For example, mutations in subunits of the succinate dehydrogenase complex have been identified in paragangliomas and pheochromocytomas, and mutations in fumarate hydratase have been associated with renal cell carcinomas and leiomyomas.^{39,70} Together with these findings, the growing body of work revealing mutations in genes encoding canonical proteins in metabolism has lent support to Otto Warburg's nearly century-old hypothesis that metabolic dysregulation could underlie tumor formation (reviewed in Kaelin,^{38,39} and in Ward and Thompson⁹⁵). The precise mechanism of tumor promotion from these mutations was unclear and awaited further study, as detailed below.

Landscape of *IDH1* Mutations in Brain Tumors

The seminal observation of recurrent *IDH1* muta-

tion in glioblastomas has been followed by significant work from many other groups that together have begun to clarify the frequency and distribution of *IDH1* mutations across all brain tumors, including gliomas and other subtypes. Many studies have extensively characterized the incidence of *IDH* mutations in brain tumors.^{4,31,33,34,77,99} Together, this work has revealed critical themes regarding recurrent mutations in *IDH1/2* loci in tumors of the CNS: 1) mutations are nearly always hemizygous; 2) mutations almost always occur at conserved codon sequences encoding a substitution at the R132 position of the *IDH1* protein; 3) *IDH1* mutation is most often identified in lower-grade (WHO Grades II and III) astrocytomas and oligodendrogliomas rather than primary glioblastomas; and 4) only a small number of *IDH2* mutations are observed in brain tumors. The R132H *IDH1* mutant is seen in more than 90% of *IDH1*-mutant tumors, but less common mutants such as R132C, R132G, R132S, and R132L have also been identified.^{31,99} Although the initial discovery of recurrent *IDH1* mutations in glioma focused on glioblastoma, data summarized from a group of studies show that only approximately 5.6% (75 of 1345) of primary glioblastomas are *IDH1* mutant^{4,6,30,33,34,77,96,99} (Table 1). In contrast, more than 76% (94 of 123) of secondary glioblastomas, which are currently defined as Grade IV astrocytomas diagnosed in patients with antecedent histological evidence of a prior lower-grade astrocytoma diagnosis, carry the *IDH1* mutation.^{4,6,33,34,62,77,96,99} In contrast to primary glioblastoma, lower-grade tumors carry a high incidence of *IDH1* mutations; this gene is mutated in more than 75% of Grade II^{4,31,33,34,77,96,99} and 62% of Grade III gliomas,^{4,30,31,33,34,77,96,99} approximately 80% of Grade II and 70% of Grade III oligodendrogliomas,^{4,6,31,33,34,77,96,99} and approximately 80% of Grade II and nearly 70% of Grade III oligoastrocytomas.^{4,31,33,34,77,96,99} Other CNS tumors found to harbor *IDH1* mutations include gangliogliomas, giant cell glioblastomas, and primitive neuroectodermal tumors, although small numbers of these tumors have been studied.⁴

Together, given the high incidence of *IDH1* mutation in LGG and secondary glioblastoma and the comparatively low incidence in primary glioblastoma, it seems plausible that *IDH1*-mutant primary glioblastomas may in fact represent misdiagnosed secondary tumors arising from previously undiagnosed LGGs—therefore suggesting that primary glioblastoma should be considered separately as a purely *IDH1*-WT disease.

In contrast to *IDH1* mutations, mutations in the *IDH2* gene are comparatively rare in gliomas. These mutations usually confer an amino acid substitution at arginine 172 of the *IDH2* protein. Of 360 primary glioblastomas in which the *IDH2* gene was evaluated, only 1 was found to harbor a mutation^{30,99} (Table 1). Whereas *IDH1* mutations are found in the majority of lower-grade astrocytomas and oligodendrogliomas, fewer than 2% of Grades II and III astrocytomas carry *IDH2* mutations, and no more than 6% of oligodendrogliomas and oligoastrocytomas are *IDH2*-mutant subtypes.^{4,99}

Additional Features Associated With *IDH1* Mutation

Although the similar incidence of *IDH1* mutation

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TABLE 1: Literature review of frequency of *IDH1* and *IDH2* mutations in CNS and non-CNS human cancers

Tumor Categories	<i>IDH1</i> Mutations	<i>IDH2</i> Mutations
tumors of the CNS		
astrocytoma		
pilocytic (WHO Grade I)		
Balss et al., 2008	1 of 41	
Ichimura et al., 2009	0 of 38	
Jiao et al., 2012	0 of 9	0 of 9
Yan et al., 2009	0 of 21	0 of 21
total	1 of 109 (0.01%)	0 of 30 (0%)
diffuse glioma (WHO Grade II)		
Hartmann et al., 2009	165 of 227	2 of 227
Ichimura et al., 2009	13 of 22	
Sanson et al., 2009	10 of 12	
Watanabe et al., 2009	60 of 68	
Yan et al., 2009	25 of 30	2 of 30
total	273 of 359 (76%)	4 of 257 (1.6%)
anaplastic (WHO Grade III)		
Hartmann et al., 2010	87 of 145	
Hartmann et al., 2009	146 of 228	2 of 228
Ichimura et al., 2009	32 of 62	
Sanson et al., 2009	9 of 18	
Watanabe et al., 2009	21 of 27	
Yan et al., 2009	36 of 52	2 of 52
total	331 of 532 (62.2%)	4 of 280 (1.4%)
primary glioblastoma (WHO Grade IV)		
Balss et al., 2008	7 of 99	
Bleeker et al., 2009	11 of 94	
Hartmann et al., 2010	17 of 237	1 of 237
Ichimura et al., 2009	6 of 173	
Nobusawa et al., 2009	14 of 377	
Sanson et al., 2009	11 of 183	
Watanabe et al., 2009	3 of 59	
Yan et al., 2009	6 of 123	0 of 123
total	75 of 1345 (5.6%)	1 of 360 (0.003%)
secondary glioblastoma (WHO Grade IV)		
Balss et al., 2008	7 of 8	
Bleeker et al., 2009	11 of 15	
Ichimura et al., 2009	5 of 10	
Nobusawa et al., 2009	22 of 30	
Sanson et al., 2009	10 of 13	
Watanabe et al., 2009	28 of 34	
Yan et al., 2009	11 of 13	0 of 13
total	94 of 123 (76.4%)	0 of 13 (0%)
oligodendroglioma		
WHO Grade II		
Hartmann et al., 2009	105 of 128	
Ichimura et al., 2009	23 of 34	
Sanson et al., 2009	41 of 54	
Watanabe et al., 2009	31 of 39	

(continued)

TABLE 1: Literature review of frequency of *IDH1* and *IDH2* mutations in CNS and non-CNS human cancers (*continued*)

Tumor Categories	<i>IDH1</i> Mutations	<i>IDH2</i> Mutations
tumors of the CNS (<i>continued</i>)		
oligodendroglioma (<i>continued</i>)		
WHO Grade II (<i>continued</i>)		
Yan et al., 2009	41 of 51	2 of 51
total	241 of 306 (78.8%)	8 of 179 (4.5%)
WHO Grade III		
Bleeker et al., 2009	1 of 2	
Hartmann et al., 2009	121 of 174	9 of 174
Ichimura et al., 2009	12 of 20	
Sanson et al., 2009	24 of 49	
Watanabe et al., 2009	6 of 8	
Yan et al., 2009	31 of 36	3 of 36
total	195 of 289 (67.5%)	12 of 210 (5.7%)
oligoastrocytoma		
WHO Grade II		
Hartmann et al., 2009	62 of 76	1 of 76
Ichimura et al., 2009	10 of 20	
Jiao et al., 2012	17 of 18	1 of 18
Sanson et al., 2009	26 of 34	
Watanabe et al., 2009	16 of 17	
Yan et al., 2009	3 of 3	
total	134 of 168 (79.8%)	2 of 94 (2.1%)
WHO Grade III		
Hartmann et al., 2009	117 of 177	11 of 177
Ichimura et al., 2009	18 of 23	
Jiao et al., 2012	21 of 22	1 of 22
Sanson et al., 2009	34 of 54	
Watanabe et al., 2009	10 of 14	
Yan et al., 2009	7 of 7	
total	207 of 297 (69.7%)	12 of 199 (6%)
non-CNS tumors		
AML		
Figuerola et al., 2010	24 of 385	33 of 385
Marcucci et al., 2010	49 of 358	69 of 358
Mardis et al., 2009	16 of 188	
Paschka et al., 2010	61 of 805	70 of 805
Schnittger et al., 2010	93 of 1414	
Ward et al., 2010	6 of 78	12 of 78
total	249 of 3228 (7.7%)	184 of 1626 (11.3%)
intrahepatic cholangiocarcinomas		
Borger et al., 2012	8 of 62	
Wang et al., 2012	22 of 325	11 of 325
total	30 of 387 (7.8%)	11 of 325 (3.3%)
central/periosteal cartilaginous tumors		
Amary et al., 2011	74 of 145 (51%)	7 of 145 (4.8%)

in lower-grade astrocytic and oligodendroglial tumors points to the possibility of shared or overlapping developmental programs, cancer sequencing studies have revealed that additional molecular features characterize

these histologically distinct *IDH1*-mutant neoplasms (reviewed in Jones et al.³⁷). The *IDH1*-mutant astrocytomas often feature both *IDH1* and *TP53* mutations, whereas the majority of oligodendrogliomas feature the combination

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of *IDH1* mutation and concurrent 1p and 19q loss of heterozygosity.^{9,34,96} Although the targets of 1p and 19q loss were elusive for many years, recent work has identified candidate genes mutated within these chromosomal regions. Bettgowda et al.⁵ used whole-exome sequencing to identify novel recurrent mutations in the *homolog of Drosophila capicua* (*CIC*) and the *far-upstream element* (*FUSE*) *binding protein* (*FUBP1*) in oligodendrogliomas. When examining the findings of Bettgowda et al. together with additional work,^{34,76,100} mutations in *CIC* and *FUBP1* are found in up to 50% and 30%, respectively, of oligodendrogliomas.³⁴ Interestingly, oligoastrocytomas harbored a low incidence of *CIC/FUBP1* mutations but a high incidence of *alpha thalassemia/mental retardation syndrome X-linked* (*ATRX*) alterations, which are also observed in the vast majority of low-grade *IDH*-mutant astrocytomas.^{32,34} Importantly, clusters of co-occurring mutations appear to define 2 distinct core *IDH1*-mutant glioma subtypes that have markedly different clinical outcomes. Specifically, *IDH1/CIC/FUBP1*-mutant tumors correlate with oligodendroglial histological features, whereas *IDH1/ATRX/TP53*-mutant tumors are typically Grades II and III astrocytomas and secondary glioblastomas.³⁴ Further work will be necessary to determine the relative impact of molecular genetics on the histological diagnoses and subsequent treatment options of these tumor types.

Mutations of IDH in Other Neoplasms

Despite extensive searches,⁴² recurring mutations in the *IDH1/2* genes seem to be restricted to select malignancies. Mutations in either *IDH1* or *IDH2* are found in approximately 15%–33% of AMLs,^{28,57,58,66,80,94} with *IDH2* mutations more common, as well as a smaller percentage of myelodysplastic syndromes.⁴⁵ Mutations tend to occur in cytogenetically normal leukemias, and mutations in both genes are usually not found in the same patient. Additionally, *IDH1/2* mutations occur in approximately 10%–12% of intrahepatic cholangiocarcinomas,^{8,93} as well as nearly 60% of central and periosteal cartilaginous tumors,¹ with *IDH1* mutations predominating in both contexts. In both of these tumor types, the R132C mutation predominated (compared with the R132H mutant in gliomas).

Somatic mosaic *IDH1/2* mutations were recently found to be the likely genetic basis of Ollier disease and Maffucci syndrome.^{2,64} These rare, nonfamilial conditions, both characterized by the early development of multiple cartilaginous tumors, have also been reported to manifest concomitant glioma or AML, thereby providing an intriguing demonstration of a potential causal role that mutant *IDH1/2* plays in these 3 distinct tumor types.⁷¹

Germline Mutations in IDH Genes

Mutations of *IDH2* were recently found in 50% of patients with D-2-HGA,⁴⁷ a rare inherited neurometabolic disorder. There are 3 general classes of 2-HGAs: D-2-HGA; L-2-HGA; and combined D,L-2-HGA.⁴⁶ Type I D-2-HGA is associated with loss-of-function mutations in the *D-2-hydroxyglutarate dehydrogenase* (*D2HGH*)

gene, which encodes the enzyme that metabolizes D-2-HG to α -KG. Thus, D-2-HG accumulates to supraphysiological levels because basal levels are not cleared. Patients with D-2-HGA without *D2HGH* mutations harbor *IDH2* mutations that encode R140 substitutions.⁴⁷ Unlike Type I D-2-HGA, D-2-HG—the same enantiomer that is seen in *IDH*-mutant tumors—accumulates due to the neomorphic function of the *IDH2*-R140 enzyme, which is reviewed in greater detail below. Patients with D-2-HGA exhibit epilepsy, psychomotor retardation, and overall hypotonia. L-2-HGA, caused by mutations in *L2HGH* and associated with elevated levels of the L enantiomer of 2-HG, is characterized by epilepsy, cerebellar dysfunction, and developmental delay. The rare combined D,L-2-HGA is a more severe neurometabolic condition whose genetic basis has not yet been identified. In these conditions, it has not yet been established whether patients with 2-HGA develop a higher incidence of gliomas or AML.

Molecular and Cellular Biology of *IDH* Mutation

Protein Functions of WT IDH

Although a clear causal mechanism of tumor promotion has not yet been fully clarified in *IDH1/2*-mutant tumors, substantial progress has been made in understanding the novel functions of mutant *IDH* proteins in cancer. Although both WT *IDH1* and *IDH2* catalyze the NADP⁺-dependent oxidation of isocitrate to α -KG, these enzymes probably do not have completely overlapping functions (reviewed in Reitman and Yan⁷²). Located in the cytosol and peroxisome, *IDH1* influences glucose sensing⁷⁴ and lipid oxidation.⁸⁸ Under hypoxic conditions, WT *IDH1* also drives lipogenesis by reducing glutamine to α -KG.⁶⁰ Located in the mitochondria, *IDH2* is a vital component of the TCA cycle critical in sugar, lipid, and amino acid metabolism.⁷⁰ Because *IDH1* and *IDH2* produce α -KG and NADPH, both proteins have also been implicated in host defense functions against insults including oxidative stress via NADPH-mediated reduction of glutathione, ultraviolet irradiation,⁵⁰ heat shock,⁸¹ and exposure to pro-inflammatory cytokines.⁴³

Biological Consequences of Mutant IDH Proteins: Neomorphic Production of D-2-HG

The major finding underlying our current thinking on mutant *IDH1/2* function was the discovery that these mutant enzymes gain the ability to catalyze a neomorphic function. Dang et al.²⁰ used unbiased liquid chromatography-mass spectrometry profiling of WT-*IDH1*-expressing and mutant *IDH1*-R132H-expressing glioma cells to show that mutant-expressing cells expressed high levels of the metabolite 2-HG. Thus, rather than catalyze the NADP⁺-dependent production of α -KG, mutant *IDH* proteins catalyze the NADPH-dependent reduction of α -KG to produce only the D stereoisomer (or “R”-enantiomer) of 2-HG (Fig. 1). Although 2-HG is also found in normal cells and can be catalyzed by WT *IDH1*,⁶⁸ its levels are exponentially higher in mutant cells—more than 100-fold in many cases. Primary *IDH1*-mutant gliomas contain extremely high levels of 2-HG,²⁰ which is not present in the

serum of patients with the *IDH1* mutation¹² but is found in the serum of patients with AML whose tumors have the *IDH* mutation.^{29,94} It is hypothesized that substitution of the R132 residue of IDH1, or the analogous R172 or R140 residue of IDH2, alters the active site such that mutant enzymes bind the carboxylate residues of isocitrate less efficiently than WT enzymes and simultaneously gain a relatively higher affinity for α -KG and the cofactor NADPH.^{20,68} Whereas initial work postulated that IDH1-R132H functioned as a dominant negative component of IDH1-WT/IDH1-R132H heterodimers,¹⁰² subsequent work has argued that these subunits probably act independently even as WT or mutant heterodimers⁶⁸ and that dominant negative properties of the mutant enzyme have not been observed at physiological concentrations of isocitrate.³⁶

Phenotypes of 2-HG–Mediated Inhibition of Dioxygenase Superfamily Members: DNA Hypermethylation

Work on the mechanistic consequences of elevated 2-HG production has converged on the observation that 2-HG inhibits the function of the α -KG–dependent superfamily of dioxygenases, with some exceptions (Fig. 1). These enzymes have diverse functions that modulate a range of cellular programs that include hypoxic sensing, histone demethylation, demethylation of hypermethylated DNA, fatty acid metabolism, and collagen modification.⁵² There is a precedent from studies of cancers with mutations in succinate dehydrogenase subunits or fumarate hydratase that the accumulation of the normal metabolic substrates for these encoded enzymes leads to the inhibition of α -KG–dependent enzymes including prolyl hydroxylases and histone demethylases.³⁸ Likewise, the inhibition of α -KG–dependent dioxygenases by 2-HG is the leading mechanism for mutant IDH1-mediated pathobiology in cancer. First, a group of studies has demonstrated increases in the hypermethylation of both DNA and also histones that are linked to the inhibition of the biologically relevant dioxygenases. By profiling the DNA hypermethylation status of glioblastomas in The Cancer Genome Atlas as well as mutant LGGs, *IDH1*-mutant gliomas were found to exhibit a global DNA hypermethylation state, termed G-CIMP.⁶³ The G-CIMP⁺ gliomas were enriched in the proneural transcriptomic subtype,^{67,90} the subtype in which *IDH1*-mutant tumors are found, and G-CIMP status was associated with improved survival. Interestingly, there is a subset of genes with both hypermethylated promoter regions and concomitant decreased expression between proneural G-CIMP⁺ and proneural G-CIMP[−] tumors. Figueroa et al.²⁷ also identified a DNA hypermethylation phenotype in *IDH1/2*-mutant AML and provided genetic evidence to link this finding to the α -KG–dependent TET2 enzyme, which converts 5-methylcytosine to 5-hydroxymethylcytosine during the process of DNA demethylation. Of 300 AML samples examined, 58 (19%) harbored *IDH1/2* mutations and 28 (9.3%) harbored *TET2* mutations; however, these were completely mutually exclusive, suggesting that these mutants conferred mostly overlapping effects. Moreover, *IDH2*-mutant-expressing murine hematopoietic cells exhibited increased numbers of immature cell populations,

raising the possibility that mutant IDH1/2 proteins influence the hierarchical progression of cellular development.

Whereas additional experiments showed that ectopic mutant *IDH1/2* expression or introduction of 2-HG into WT cell models led to increased 5-methylcytosine levels^{27,98} also observed in glioma specimens,⁹⁸ Turcan et al.⁸⁷ provided definitive data to show that mutant *IDH1* was the cause of the G-CIMP phenotype. Introduction of mutant IDH1 into immortalized normal human astrocytes⁸⁴ recapitulated the hypermethylated epigenomic landscape of the G-CIMP phenotype that was also observed in *IDH1*-mutant LGGs. Additionally, the transcriptional signature seen in the *IDH1* mutant-engineered astrocyte system was concordant with that observed in *IDH1*-mutant gliomas, providing further evidence that mutant IDH1 was largely responsible for the transcriptional and epigenetic landscapes seen in mutant tumors. Interestingly, the density of DNA methylation increased with repeated passages, suggesting that remodeling of the epigenome is not an immediate process. Ultimately, to demonstrate the tumor promotion of 2-HG-induced epigenomic changes of the genes identified to be hypermethylated and differentially expressed between *IDH1*-WT and *IDH1*-mutant cells, it will be necessary to determine which, if any, are crucial to tumor formation and/or maintenance.

Histone Hypermethylation

The epigenomic landscape of *IDH*-mutant cells is also probably influenced by the increased methylation of histone proteins. Histones are central to the structure of eukaryotic DNA, which is composed of nucleosomes of approximately 146-bp stretches of DNA wrapped around octamers of 4 histone proteins (H2A, H2B, H3, and H4).²¹ Histone protein tails are frequent sites for modifications such as covalent methyl group addition. The area of histone modifications within epigenetic biology is rapidly evolving and is well reviewed elsewhere.^{21,89,101} Several groups have found that histone demethylases are inhibited by the 2-HG metabolite.^{18,53,87,98} Chowdhury et al.¹⁸ showed that (R)-2-HG inhibited recombinant forms of the JMD2A, JMJD2C, and JHDM1A/FBXL11 histone demethylases and that the JHDM1A/KDM2A and KDM5B/JARID1B/PLU-1 demethylases were also inhibited by 2-HG treatment.⁹⁸ Further studies have documented the specific histone methylation sites that are modified in the setting of 2-HG treatment and/or mutant IDH expression. Specifically, increases in methylation in response to 2-HG or mutant IDH1 expression were observed in H3K4me1,⁹⁸ H3K4me3,^{53,98} H3K9me2,^{53,98} H3K27me2,⁹⁸ H3K79me2,^{87,98} H3K27me3,^{53,87} H3K9me3,⁵³ and H3K36me3.⁵³ Furthermore, H3K79me2,⁹⁸ H3K9me3, and H3K27me3⁵³ histone marks are increased in *IDH1*-mutant glioma samples. Although it is tempting to conclude that increased histone methylation is associated with heterochromatic states and repressed gene expression, the picture is known to be more complex. Generally, H3K9 and H3K27 methylation is seen in gene silencing, whereas the methylation of H3K4, H3K36, and K3K79 is seen in gene expression,⁸⁹ although this classification is probably an oversimplification given the additional components involved in chromatin remodeling.

In the study by Lu et al.,⁵³ H3K9 modification—in particular H3K9 trimethylation (H3K9me3)—was signif-

Fig. 1. Schematic depicting the multiple functions of the IDH1-mutant enzyme. The WT IDH1 and IDH2 enzymes normally catalyze the production of α -KG from isocitrate in the cytosol/peroxisomes and the mitochondria, respectively. Mutant IDH1 exhibits a neomorphic function by catalyzing the production of the (D/R)-enantiomer of 2-HG. Very high levels of this putative oncometabolite inhibit a range of α -KG-dependent dioxygenases. The inhibition of TET2 hydroxylases probably leads to increased DNA hypermethylation, and inhibition of histone demethylases leads to increased histone tail methylation. Together, these covalent modifications modify the epigenome to influence transcriptional programs and cellular developmental programs. The 2-HG metabolite also inhibits prolyl hydroxylases, which have been shown to inhibit collagen maturation in a process that can induce an unfolded protein response. The 2-HG metabolite also influences HIF biology; inhibition of prolyl hydroxylases may lead to increased HIF levels and downstream programs such as angiogenesis, whereas stimulation of prolyl hydroxylases can attenuate the HIF response and lead to cellular transformation. CoA = coenzyme A; ER = endoplasmic reticulum; VEGF = vascular endothelial growth factor.

icant when mutant IDH1 was expressed in immortalized astrocytes. As is the case with DNA hypermethylation, this histone mark progressively increased with increasing cell passage. Further work linked impaired cell differentiation with mutant IDH1 expression and histone methylation. Expression of mutant IDH1 impaired the differentiation of 3T3 cells into adipocytes, as did the knockdown of the H3K9 demethylase KDM4C. Furthermore, mutant IDH1-expressing murine neurospheres failed to express GFAP compared with WT neurospheres when exposed to retinoic acid. However, no clear link was established between mutant IDH1 expression and histone demethylation in this process.

Taken together, it is becoming clear that *IDH1/2*-mutant cells undergo changes in the epigenetic landscape that involve DNA and histone methylation, which probably leads to chromatin remodeling. At this point, it is unclear which enzymes are most critical for these processes, which chromatin modification readers modulate these changes, and what the long-term effects on gene expression programs might be. Further clarification of these

mechanisms will be important to determine which nodes may represent potential therapeutic targets.

Effects on HIF-1 α Biology

In addition to effects on the epigenome, mutant IDH1 proteins appear to influence the biology of the HIF. However, unlike the largely concordant studies on the epigenomic effects of the mutant IDH proteins, work on mutant IDH1 and HIF suggests that this relationship is complex and will require further clarification. First, 2-HG may stabilize HIF-1 under some conditions.^{98,102} Ectopic expression of mutant IDH1 or 2-HG treatment in U87 malignant glioma cells led to increased HIF protein levels, which were also modestly increased in the CNS-specific *IDH1*-mutant mouse model that is discussed in more detail below.⁷⁸ However, IDH1-R132H and HIF expression were not entirely concordant when assessed using immunohistochemistry in glioma tissue.⁹⁷ Although key biological variables are difficult to quantify in a post hoc analysis of fixed, paraffin-embedded tissue, this finding does underscore that further work is needed to un-

derstand the effects on HIF biology of mutant protein expression. A recent study provided evidence to support an alternative role for HIF in *IDH1*-mutant tumors. Kivunien et al.⁴⁴ showed that expression of IDH1-R132H in the same immortalized human astrocyte model system⁸⁴ used in the study by Turcan et al.⁸⁷ led to more colonies formed in soft agar.

Furthermore, mutant IDH1 protein expression was correlated with decreased HIF levels; although HIF protein levels appeared to increase with cell passage, the hypoxia-induced increase in HIF was completely abolished in *IDH1*-expressing astrocytes, HCT116 cells expressing physiological mutant IDH1 levels via knock-in technology, or *IDH1*-mutant gliomas derived from patient samples. Moreover, HIF gene signatures were downregulated in *IDH1*-mutant tumors analyzed in the data set in The Cancer Genome Atlas. Interestingly, whereas this study showed that D-2-HG inhibited some of the dioxygenases previously discussed—such as the TET1 and -2 hydroxylases, HIF asparaginyl hydroxylase, and JMJD2D/KDM4D histone demethylase—D-2-HG stimulated the HIF prolyl 4 hydroxylase EGLN1. Furthermore, knockdown of HIF alone was sufficient to increase soft agar growth of engineered normal human astrocytes, and EGLN1 was both necessary and, by itself, sufficient to drive similar in vitro colony formation. It is not clear what accounts for the differences in effects of mutant IDH1 protein expression on HIF levels across these recent studies, although differential expression or alterations in the EGLN prolyl hydroxylases could influence this biology across different in vitro model systems. Further study will be necessary to characterize the mutant IDH1–HIF1 relationship fully and, in particular, to clarify further the HIF-dependent contributions to the tumor microenvironment.

Cell-Based Models of Mutant IDH1 Protein

Until recently, there has been a dearth of human cell lines that carry endogenous rather than ectopically introduced *IDH1* mutations. Engineered systems such as immortalized normal human astrocytes expressing IDH1 have been tremendously useful in studying the causal effects of mutant protein expression,^{53,87} but the caveat remains that these lines do not harbor naturally arising somatic *IDH1* mutations. One well-established mutant cell line, the HT1080 fibrosarcoma cell line expressing a naturally arising mutant IDH1-R132C protein, was used recently in the only study to date that has demonstrated the dependency of an established *IDH1*-mutant cell line on persistent mutant protein expression.³⁵ Selective knockdown of mutant IDH1 led to decreased proliferation and clonogenic capacity of the parental cell line, suggesting that mutant cells remain dependent on the expression of mutant IDH1 protein and providing a rationale that inhibiting this enzyme may have therapeutic benefit. The *IDH1*-mutant tumor-initiating cell models, or neurospheres, have traditionally been difficult to develop but have recently been reported.⁵⁴ A neurosphere line from a patient with *IDH1*-mutant anaplastic oligodendroglioma survived passage in vitro, was transplanted as a xenograft in vivo, and generated the expected 2-HG. Further large-scale development of these patient-derived models will be

critical to generalize the dependency of these tumors on mutant protein expression and will undoubtedly serve as important preclinical models.

Animal Models of Mutant IDH1 Protein

Recent generation of genetically engineered mouse models of the *IDH1-R132H* mutation has provided additional insights into the physiologically relevant consequences of mutant IDH1 protein expression. Both studies are from the Mak group (see Sasaki and colleagues^{78,79}) and use lineage-specific *Cre* recombinase-driven knock-in technology. In this approach, a mutant *IDH1-R132H* gene harboring an upstream transcriptional stop site flanked by *Cre* recombination lox sites is introduced by homologous recombination into the endogenous WT *IDH1* locus. When these heterozygously targeted mice are bred to transgenic mice expressing *Cre* under distinct tissue-specific promoters, the stop site upstream of the mutant *IDH1* gene is deleted, and mutant protein is expressed at physiological levels. To study the expression of IDH1-R132H in the brain, Nes-KI mice were generated in which mutant *IDH1* was expressed in the neural stem cell compartment as early as embryonic Days 10.5–12.5.⁷⁸ Surprisingly, these mice were born at the expected mendelian frequency, but all died in the immediate perinatal period after birth. Necropsy of Nes-KI mice revealed uniform massive cerebral and cerebellar hemorrhage that was present in embryos prior to birth and was associated with massive apoptosis.

Three findings provided in vivo evidence for 2-HG-mediated inhibition of several dioxygenases, although not all previously reported findings in humans were observed. First, Nes-KI brains had increased levels of immature Type IV collagen that may contribute to an endoplasmic reticulum stress response, suggesting that the prolyl hydroxylases that hydroxylate these collagen forms were inhibited. Second, Nes-KI brains displayed increased levels of 5-hydroxymethylcytosine, consistent with inhibition of the TET2 hydroxylases. However, it is unclear whether this model recapitulates the extent of the global G-CIMP phenotype found in human *IDH1*-mutant tumors.⁶³ Third, the expression of HIF-1 α protein and HIF-1 α -regulated genes was increased in Nes-KI mice, consistent with inhibition of the prolyl hydroxylases that destabilize HIF-1 α . Histones were not found to be hypermethylated, although it is unclear whether this finding may have been masked as a result of insufficient time for histone marking due to perinatal lethality. Although the Nes-KI mice died too early to assess tumor formation, GFAP-KI mice were also generated in which the *IDH1-R132H* cassette was probably expressed in neurons, glia, and some neuronal precursor cells. However, GFAP-KI mice did not develop tumors, and attempts to breed GFAP-KI mice onto a *p53*-deficient background proved difficult. It will be important to develop additional models in which mutant IDH1 is expressed conditionally/inducibly in relevant *p53*-, *ATRX*-, and/or *CIC*-deficient backgrounds to develop a faithful model of *IDH1*-mediated glioma development.

Sasaki et al.⁷⁹ also modeled *IDH1*-mutant AML by generating mice in which mutant IDH1 was expressed in all hematopoietic cells (Vav-KI mice) or in the myeloid lineage (LysM-KI mice). Unlike the CNS models, these

Mutant *IDH1/2* in glioma

mice were born at expected mendelian frequencies and had normal lifespans. Although the resulting phenotype provided critical insight into the mechanistic basis of mutant *IDH1* function, these mice did not develop leukemia. As the animals aged, both Vav-KI and LysM-KI mice developed anemia and splenomegaly and accumulated increased numbers of immature cell populations, although there was no clear block in their ability to differentiate. Importantly, immature cells from LysM-KI mice displayed a DNA CpG and histone hypermethylation phenotype strikingly similar to that found in human patients with AML. However, HIF target genes were not upregulated, suggesting that the downstream effects of 2-HG-mediated modulation of the dioxygenase superfamily may be influenced by tissue context. Further study of these models should clarify the biological programs critical for mutant *IDH1*-mediated tumor development, especially if current models are bred onto backgrounds with additional cancer-relevant lesions.

Taken together, these data from studies of mutant *IDH1/2* biology suggest that the expression of mutant enzymes influences a broad range of biological programs. However, especially in a setting wherein the production of 2-HG has the potential to exert pleiotropic effects on a large class of enzymes, it will be crucial to distinguish what can happen from what actually does happen to initiate and/or maintain the tumor phenotype by using physiologically relevant cell-based models.

Mutations in IDH1/2 Are Early Events in Brain Tumorigenesis

Several recent studies have provided evidence that mutations in *IDH1/2* represent early events in brain tumor formation. Watanabe et al.⁹⁶ focused on the presence of the *IDH1* mutation, *TP53* mutation, and 1p/19q loss in patients who had undergone multiple biopsies for Grade II or III gliomas. Interestingly, 7 patients who carried the *IDH1* mutation at first biopsy had acquired either the *TP53* mutation or 1p/19q loss at the second biopsy; there were no cases in which either *TP53* or 1p/19q loss preceded *IDH1* mutation. Although these data point to a potential temporal sequence of genetic alterations in glioma, further work in which larger sets of serial tissue samples obtained from the same patient are used will be necessary to demonstrate this possibility more definitively.

A mechanistic basis for the temporal acquisition of mutations in LGGs was proposed by Lai et al.⁴⁹ By analyzing the sequences of *TP53* and *IDH1* in a large panel of Grades II–IV astrocytomas, these authors found that there were higher rates of Arg-to-Cys substitutions at position 273 in *TP53* compared with the high rate of Arg-to-His substitutions at position 132 in the *IDH1* gene. The authors proposed that this difference was caused by a strand asymmetry mechanism⁷³ in which C→T mutations occurred on the nontranscribed DNA strand in *TP53*, and *IDH1* mutations occurred on the transcribed strand in *IDH1*. Theoretically, this difference would enable *IDH1* mutations to take place in nondividing clones prior to S-phase entry, which would be required for *TP53* alterations on the complementary DNA strand. Further work using careful clonal analysis and appropriate clinically relevant animal models will

be needed to clarify this possibility. Additionally, further in-depth analysis of serial tissue samples from the same patient will provide a deeper understanding of disease evolution and progression.

Translational Implications of Mutant *IDH1/2*

In the short time since the identification of *IDH* mutations in glioma, there has been a significant amount of work focused on the translational relevance of these mutations. It is already clear that *IDH1* status is a major determinant of survival, and many groups have developed new ways to identify the mutations from clinical samples, as well as the oncometabolite 2-HG, by using noninvasive methods. It is likely that the determination of *IDH1* status in glioma will be an early step in treatment algorithms for patients with this disease.

Clinical Consequences: Survival and Imaging Findings

Patients with *IDH1* mutations in any grade of glioma exhibit increased overall survival compared with patients with *IDH1*-WT tumors, and patients with *IDH1*-mutant glioblastomas tend to be nearly 2 decades younger, on average, than patients with *IDH1*-WT glioblastomas. The differences in overall survival between *IDH1*-mutant versus *IDH1*-WT glioblastomas across several studies was 3.8 versus 1.1 years,⁶⁵ 2.6 versus 1.3 years,⁹⁹ 2.3 versus 1.2 years,⁷⁷ and approximately 3 years versus 1 year.³⁰ Moreover, the differences in overall survival between *IDH1*-mutant versus *IDH1*-WT anaplastic astrocytomas was 5.4 versus 1.7 years,⁹⁹ 6.8 versus 1.6 years,⁷⁷ and approximately 7 versus 2 years.³⁰ This survival benefit was also observed in G-CIMP⁺ versus G-CIMP⁻ gliomas, wherein G-CIMP has a high association with *IDH1*-mutant status.⁶³ When both histological and molecular features were taken into account, patients with *IDH1*-mutant glioblastoma had a median survival of nearly 3 years, whereas patients with *IDH1*-WT anaplastic astrocytoma had a median survival of less than 2 years. Strikingly, for patients older than 60 years, overall survival was equivalent for *IDH1*-WT anaplastic astrocytomas and *IDH1*-WT glioblastomas. The survival benefit also extended to Grade II gliomas; patients with mutant tumors had a median overall survival of 12.6 versus 5.5 years.⁷⁷ Together, these data highlight the major impact *IDH1* status has on survival and support the incorporation of molecular features into histopathological assessment.

Diagnostic Evaluation of IDH1 Status

There has been rapid development and clinical application of antibody and sequencing-based methods to detect *IDH1* status. Monoclonal antibodies to *IDH1*-R132H, first described by von Deimling's group (see Capper et al.¹⁴), recognize the mutant protein with a high degree of sensitivity and specificity^{13,86} (Fig. 2). Beyond determination of mutant status, the histopathological utility of this antibody has extended to additional clinical scenarios such as the discrimination between diffuse astrocytoma and reactive astrogliosis when combined with a panel of key molecular features.^{10,11} Additional antibody reagents

Fig. 2. Photomicrographs showing mutant IDH1-specific immunohistochemistry findings in tumor sections. A monoclonal antibody recognizing the IDH1-R132H mutant protein does not stain IDH1-WT anaplastic astrocytoma (**left**) but stains nearly all cells in an IDH1-mutant tumor of the same histology (**right**). Both panels demonstrate the classic features of nuclear pleomorphism, increased cellularity, and mitotic activity found in a Grade III astrocytoma.

to the less common IDH1 mutations, such as R132S, have also been reported.⁴¹

Several technologies are currently in use to detect mutations in the *IDH1* gene. SNaPshot²² and Oncomap,⁵⁶ both of which can be used with paraffin-embedded tissue, use single base pair extension that results in an allele-specific probe that is read out by either fluorescence detection (SNaPshot) or mass spectrometry (Oncomap). Alternative approaches, such as pyrosequencing²⁶ and next-generation sequencing,⁵¹ are also in use for the detection of mutations in tumor samples. In addition to these approaches, recent work demonstrated proof of principle in detecting *IDH1* mutations from the plasma of patients with mutant gliomas.⁷ Although the potential application for monitoring disease noninvasively is compelling, the sensitivity attained using this assay was 60%. Further work on the nature of circulating tumor material will be necessary to determine whether it will be possible to monitor the *IDH1* mutation status in the peripheral blood of all patients with mutant gliomas.

Methods to Detect the 2-HG Metabolite

Another area of investigation has focused on the detection of the 2-HG metabolite rather than on the specific sequence of the *IDH1/2* gene or protein product. In theory, sensitive and specific detection of 2-HG is sequence independent in that 2-HG should be present regardless of the type of mutation in either *IDH1* or *IDH2*. High levels of 2-HG have been detected in ex vivo tissue samples by using 2 approaches. In the first, combination gas or liquid chromatography/mass spectrometry was used to identify 2-HG in frozen¹⁹ or paraffin-embedded⁷⁵ glioma tissue. However, these extraction-based approaches do not preserve the integrity of the sampled tissue. In the second approach, which is used by several groups,^{25,40} proton High-Resolution Magic Angle Spinning (¹H HRMAS) MRS is used to determine the metabolic profiles in ex vivo tissue; this technique does not require alteration of tissue samples. This method identified 2-HG in ex vivo specimens with high degrees of sensitivity and specificity. Unlike the case with AML, wherein 2-HG can be detected in the

blood of patients with *IDH*-mutant AML,⁹⁴ its presence in peripheral blood is similar between patients with *IDH1*-mutant and -WT tumors.¹²

Detection of 2-HG by MRS represents a completely noninvasive method with which to determine the presence of *IDH* mutations in gliomas, irrespective of the sequence of the mutation or whether the mutation maps to *IDH1* or *IDH2*. Importantly, this approach represents the only example in human cancer in which a genomic feature can be identified specifically by using imaging-based metabolic profiling. Prior work has demonstrated that *IDH*-mutant tumors display characteristic imaging findings. The *IDH1*-mutant tumors display reduced contrast enhancement, less surrounding edema, cystic components, and are often found in the frontal lobe compared with *IDH*-WT tumors.^{15,49} This work has been complemented by MRS-based approaches. Because it is recognized that the spectrum of 2-HG has some overlap with other commonly found metabolites such as glutamate and glutamine, the methodology for obtaining and analyzing in vivo MRS data is critical. One study to demonstrate 2-HG detection in glioma by MRS used the acquisition of a point-resolved spectroscopy (PRESS) sequence.⁶⁹ There was good concordance between sequence-based mutation status and 2-HG detection, although several false positives and false negatives were reported. Additional studies in which spectral editing analysis¹⁷ or 2D-correlation MRS with spectral editing³ were used demonstrated that *IDH* mutation status can be identified noninvasively by MRS techniques with a high level of sensitivity and specificity.

Figure 3 provides an example of the methods used by Andronesi et al.³ to detect 2-HG in glioma patients, and it demonstrates the advantages of complementing molecular identification of mutation status with imaging-based approaches. In this case of a 32-year-old patient with a non-enhancing tumor that was assessed as a WHO Grade II glioma, the tumor harbored a noncanonical IDH1-R132G mutation that was not detected by conventional anti-IDH1-R132H immunohistochemical approaches and required the additional step of DNA sequence profiling of the ex vivo tissue sample. However, the mutant status was identified unambiguously in the preoperative setting by using MRS with J-difference spectral editing (MEGA-LASER sequence³), which subtracts the signals of overlapping metabolites such as phosphocreatine, myoinositol, and lactate at or near the 4.02-ppm H α signal of 2-HG. Ultimately, imaging-based 2-HG detection may be an important method with which to determine not only the mutant status of tumors in patients before surgery but also to monitor tumor recurrence, distinguish between treatment effect or pseudoprogression, and possibly also monitor response to treatments in the event that targeted therapies are developed for *IDH*-mutant tumors. 2-HG imaging could also help better delineate margins of the active tumor compared with contrast enhancement or FLAIR hyperintensity.

Treatment Implications for IDH1-Mutant Tumors

The identification of *IDH1/2* mutations and the rapid characterization of their protein products present a therapeutic opportunity to treat these mutant tumors. From a medical standpoint, it will be important to de-

Fig. 3. Results of 3-T MRS imaging in a 32-year-old patient with an *IDH1*-mutant Grade II astrocytoma in the left parietal lobe. **A:** Choline map showing the hot spot of high tumor proliferation. **B:** Spectral grid overlaid on the anatomical T2 hyperintensity of the tumor. **C:** Example of a spectrum from a representative voxel within the tumor core showing high total choline (tCho), low creatine (Cre), lactate (Lac), and absent *N*-acetylaspartate. **D:** A J-difference (MEGA-LASER) spectrum that edits unambiguously the signal of the H α proton of 2-HG at 4.02 ppm by subtraction of the overlapping metabolites. Note that H α signals from glutamate and glutamine (Glx) and GABA plus macromolecules (GABA + MM) are also coedited at different positions. GABA = γ -aminobutyric acid.

termine whether *IDH1/2*-mutant tumors would be sensitive to small molecules that inhibit the mutant enzymes. Although there are no published studies to date addressing this possibility, a recent study suggested that mutant tumor cells may be dependent on the continued expression of the mutant enzyme and/or its resultant 2-HG metabolite. As noted previously, Jin et al.³⁵ showed that a cell line expressing endogenous mutant *IDH1* required its expression for survival and anchorage-independent growth, suggesting that pharmacological inhibition of mutant *IDH1* may recapitulate this result. With respect to mutant *IDH1*-mediated biology, this result would also suggest that 2-HG-induced cellular changes such as global DNA hypermethylation are either potentially reversible or, if not, are insufficient for tumor maintenance. Noninvasive methods of monitoring inhibitor effectiveness, such as the MRS imaging we have summarized above, would be useful in monitoring drug responses in glioma.

We anticipate that inhibition of this pathway would increase patient survival. Although there has been some discussion of whether it is prudent to inhibit mutant *IDH1* because patients with mutant tumors have a better survival than patients with WT tumors, we do not expect that inhibiting the mutant enzyme would make these tumors behave like their more aggressive WT counterparts. The differences in survival most likely stem from the fact that *IDH1*-mutant and -WT tumors arise from distinct lineages and ontogenies and thus represent entirely different neoplastic disease processes. Because gliomas acquire the *IDH1* mutation very early in their development, akin to *BRAF* mutations in dysplastic nevi and melanoma, we envisage that it similarly represents a targetable dependency.

From a surgical standpoint, it will be important to determine whether the mutation status of gliomas influences surgical management. In particular, it is possible that *IDH1* status could have implications for the benefit of surgery.

Ongoing study is directed at assessing the radiographic features of *IDH1*-mutant and *IDH1*-WT tumors that are particularly critical targets for surgical management.

Conclusions

The identification of recurrent *IDH1* mutations in glioma was a seismic discovery that has established new paradigms in cancer research and, in a short time frame, is already influencing clinical practice. From a basic research standpoint, it will be critical to determine the physiologically relevant consequences of mutant IDH1 protein function. We are beginning to understand the broad range of cellular processes that can be influenced by the production of 2-HG, but we do not yet have a clear understanding of which programs are critical for IDH1-mediated transformation and for tumor maintenance. Regarding the latter point, there are preliminary data suggesting that even established tumor cells require the continued expression of mutant enzymes, but this point must be rigorously evaluated in patient-derived mutant cell models and also disease-faithful animal models. The evaluation of persistent dependency is especially important because it is not immediately obvious that changes in the epigenetic landscape are always reversible if they are truly responsible for much of the mutant IDH phenotype. This demonstration of unequivocal and generalizable dependency of mutant tumors on mutant IDH1 expression will provide a powerful justification for accelerated efforts to develop inhibitors of the mutant enzymes, which presumably would display a large therapeutic window. The clarification of the downstream effects of mutant IDH1 expression may also reveal additional therapeutic targets. Furthermore, it is important not to overlook the fundamental mechanisms that underlie why *IDH1*-WT tumors are so formidable.

From a clinical standpoint, the status of a tumor's *IDH1* gene already gives the health care provider important prognostic information on overall survival that can and should be shared with patients. Across all grades of glioma, the presence of gene mutation predicts longer survival. It is such a critical piece of information that it is evaluated by immunohistochemistry and polymerase chain reaction–based DNA sequence analysis for all patients with glioma at our institution and in many others. We anticipate and agree with the incorporation of molecular profiling of important features such as *IDH1* status into the current histopathological grading of all gliomas.⁹² However, further analysis is necessary to determine whether *IDH1* gene mutation status should change clinical management with respect to chemotherapy/radiation and/or surgery. Nevertheless, our clinical heuristic for glioma care has already incorporated *IDH1*-WT or *IDH1*-mutant categories at the earliest stage in our approach to a patient, in large part because the biological and clinical data point to the fact that mutational status segregates otherwise histologically similar lesions into what increasingly appear to be different diseases.

Clearly, with the discovery of *IDH1* mutations in glioma in the context of the ongoing characterization of the glioblastoma genome, it is an important—and exciting—era in the field. In a short period of time, we have

moved from the phase of genomic “basic science” discovery in glioma to the cusp of the development of inhibitors to mutant IDH1 proteins that may offer clinical benefit to patients with these tumors. Ultimately, we hope that a critical take-home message gleaned from the story of mutant *IDH1* in glioma will be a powerful demonstration that a fundamental understanding of the structure of the glioma genome was pivotal to the development of clinically meaningful therapies for patients with this disease.

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