Cancer-associated mutation and beyond: The emerging biology of isocitrate dehydrogenases in human disease

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Isocitrate dehydrogenases (IDHs) are critical metabolic enzymes that catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG), NAD(P)H, and CO₂. IDHs epigenetically control gene expression through effects on αKG-dependent dioxygenases, maintain redox balance and promote anaplerosis by providing cells with NADPH and precursor substrates for macromolecular synthesis, and regulate respiration and energy production through generation of NADH. Cancer-associated mutations in IDH1 and IDH2 represent one of the most comprehensively studied mechanisms of IDH pathogenic effect. Mutant enzymes produce (R)-2-hydroxyglutarate, which in turn inhibits αKG-dependent dioxygenase function, resulting in a global hypermethylation phenotype, increased tumor cell multipotency, and malignancy. Recent studies identified wild-type IDHs as critical regulators of normal organ physiology and, when transcriptionally induced or down-regulated, as contributing to cancer and neurodegeneration, respectively. We describe how mutant and wild-type enzymes contribute on molecular levels to disease pathogenesis, and discuss efforts to pharmacologically target IDH-controlled metabolic rewiring.

Oftentimes genetically programmed, altered metabolic states disturb normal organ physiology and cause or contribute to a broad spectrum of human disease, including cancer and neurodegeneration (1, 2). Cancer has become a prime example of how genetically defined metabolic perturbation cause disease phenotypes. To fulfill bioenergetic and biosynthetic demand associated with unabated growth and survival, as first discovered by Otto Warburg more than 90 years ago, cancer cells rely on enhanced conversion of glucose to lactate of generate energy in the form of adenosine 5’-triphosphate (ATP) and to maintain carbon intermediates that fuel macromolecular synthesis (1). Other examples of metabolic rewiring of cancer cells include enhanced glutamine catabolism, heightened glucose oxidation through the tricarboxylic acid (TCA) cycle, and the rampant synthesis of fatty acids, all of which contribute to tumor progression in experimental in vitro and in vivo models of cancer (3). Similarly, metabolic alterations affect the initiation and progression of neurodegenerative disorders. Brain regions of patients affected by Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), or amyotrophic lateral sclerosis (ALS) show reduced rates of glucose utilization, which, in turn, result in diminished energy availability and increased vulnerability to cognitive impairment and dementia (4). On a molecular level, expression of glucose transporters and activity of several enzymes implicated in glycolysis, pentose phosphate pathway, and TCA cycle turnover are altered, resulting in glucose hypometabolism and mitochondrial dysfunction (5).

Because of their central role in energy metabolism, redox balance, and anaplerosis, metabolism research over the past decade has focused on elucidating mechanisms of catalysis, enzyme structure, regulation, and role in organismal homeostasis, more recent studies aimed to understand how IDH activity, when altered through mutation or transcriptional deregulation, contributes to disease. Here, we review the spectrum of human diseases associated with altered IDH activity, describe physiological and pathophysiological mechanism of IDH metabolic effect, discuss strategies to target IDH metabolic rewiring, and point to future lines of research to more fully understand the complex biology of IDHs in health and disease.

THE IDH FAMILY—ENZYME STRUCTURE, CATALYTIC MECHANISM, AND ALLOSTERIC REGULATION

Eukaryotic cells express three IDH paralogs, which differ in subcellular localization, structural organization, cofactor requirement, allosteric regulation, and catalytic mechanism (Fig. 1) (6). While all three IDH enzymes catalyze the oxidative decarboxylation of isocitrate (ICT) to α-ketoglutarate (αKG), their roles in cellular metabolism are overlapping but nonredundant. IDH1 localizes to both cytosol and the peroxisomes, while IDH2 and IDH3, as part of the TCA cycle, are found within the mitochondrial matrix. IDH1 and IDH2 function as homodimers, use NADP⁺ (nicotinamide adenine dinucleotide phosphate) as electron acceptor, and require binding to a divalent metal ion, typically Mn²⁺ or Mg²⁺. Each IDH1 subunit is composed of three domains: a large domain (residues 1 to 103 and 286 to 414), a small domain (residues 137 to 185) (7), (8). The active site, formed by the large and small domains of one subunit and a small domain of the other subunit, includes the NADP⁺-binding and the ICT-metal ion-binding site. To initiate substrate binding, one of the subunits adopts an asymmetric open conformation, while the other adopts a quasi-open conformation. The binding of ICT subsequently induces a closed conformation, which results in the formation of the catalytically active enzyme (8), Bacterial IDH is reversibly regulated by phosphorylation of Ser113. Although the residue is conserved in the mammalian protein [Ser91; (7)], no evidence of phosphorylation of any mammalian IDH has been reported. Instead, mammalian IDH1 controls substrate
binding through a self-regulatory mechanism. In the inactive enzyme configuration, Asp\(^{379}\) occupies the ICT-binding site and forms hydrogen bonds with Ser\(^{94}\). ICT breaks the hydrogen bonds between these residues and enables Asp\(^{379}\) to chelate the metal ion in the active enzyme configuration (7).

IDH2 forms a homodimer, which, similarly to IDH1, consists of three domains (i.e., a large domain, a small domain, and a clasp domain) and adopts a closed conformation for full enzymatic activity (9). A hydrophilic cleft formed between the large domain and the small domain serves as the active site. Arg\(^{140}\) and Arg\(^{172}\), frequently mutated in cancer (see below), together with Arg\(^{149}\) and Lys\(^{256}\), stabilize the ICT-binding pocket (9). In particular, analysis of the IDH2 crystal structure revealed that Lys\(^{256}\) electrostatically repels a lysine-rich cluster on the opposing site of the active center and, in so doing, keeps the substrate binding pocket accessible for and enables binding of ICT. Consistent with this model, Lys\(^{256}\) acetylation results in less electrostatic repulsion and, consequently in a narrower binding pocket, and reduced IDH2 activity (9). Together with additional reports that identified mitochondrial sirtuins as IDH2 deacetylators (10), these studies point to acetylation/deacetylation as a critical mode of IDH2 regulation.

IDH3 is a heterotetramer composed of two 37-kDa α subunits (IDH3α), one 39-kDa β subunit (IDH3β), and one 39-kDa γ subunit (IDH3γ) (11). The subunits are encoded by the IDH3A, IDH3B, and IDH3G genes, respectively. IDH3 catalyzes an irreversible and rate-limiting step in the TCA cycle, which is tightly regulated through substrate availability [citrate, adenosine 5'-diphosphate (ADP), ICT, nicotinamide adenine dinucleotide (NAD\(^+\)), and Mg\(^{2+}\)/Mn\(^{2+}\)]. The catalytic domain of the holoenzyme (12), and molybdenum (Mo), is required for full activity of the holoenzyme (13). While the crystal structure of the IDH3holoenzyme has not been reported yet, systematic biochemical and mutagenesis studies revealed that α and γ subunits form the metal–ICT binding and α and β subunits mediate NAD\(^+\) binding (11, 12). When coexpressed, αβ and αγ complexes have considerable basal activity; cooperative function of both heterodimers, however, is required for full activity of the holoenzyme (12).

**IDHs and the pleiotropic effects of αKG**

IDHs through production of αKG promote the activity of αKG-dependent dioxygenases that epigenetically control gene expression. These enzymes catalyze a broad array of biochemical processes and reactions, resulting in the chemical modification of DNA, RNA, protein, and lipids. While the pleiotropic functions of dioxygenases have been reviewed in detail elsewhere (13), we will highlight key enzymatic activities that affect cancer progression in response to cellular αKG.

Histones are frequently methylated at Lys and Arg residues, resulting in DNA supercoiling, and modulate gene expression through alterations in overall chromatin conformation (14). Jumonji (Jm) catalytic domain containing N-methyl-lysine and N-methyl-arginine demethylases represent a large group of oxygenases that remove methyl groups from histones and control gene expression signatures linked to the pathogenesis of many different types of cancer (15). The activity of JmJ histone demethylases appears to be tissue type specific, with many enzymes acting as tumor suppressors or oncogenes depending on cellular context. Consistent with tumor-suppressive function, certain JmJ demethylases repress oncogenic and activate tumor suppressor signaling, and are expressed at lower levels in certain tumor types compared to normal tissue, such as KDM3B, which is frequently deleted in myelodysplastic syndromes (MDSs) and acute myeloid leukemia (AML) characterized by 5q chromosomal deletion (15).

DNA demethylation, which occurs at CpG islands interspersed throughout the genome, is facilitated by αKG-dependent oxygenases belonging to the ten–eleven translocation methylcytosine dioxygenase (TET) family of enzymes. TET enzymes hydroxylate 5-methylcytosine (5mC) to generate 5-hydroxymethylcytosine (5hmC), which can be further oxidized to 5hmC, resulting in the generation of 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) (16). Inactivating mutations in TET2 frequently occur in AML, MDS, myeloproliferative neoplasms (MPNs), and chronic myelomonocytic leukemia (CML) and, as described below, are mutually exclusive with IDH mutations (17). Reflecting their differential expression throughout development, TET enzymes regulate pluripotency and differentiation potential of embryonic stem and, when deregulated in cancer, affect differentiation of lineage-specific progenitor cells (18). In addition to DNA modification, TET enzymes together with other αKG-dependent oxygenases, such as αKG-dependent dioxygenase alkB homolog 3 (ALKBH3) and fat mass and obesity-associated protein FTO, can demethylate mRNA to enhance mRNA stability and translation (17).

Eight groups of αKG-dependent oxygenases can catalyze the hydroxylation of proteins, including collagen hydroxylases, epidermal growth factor (EGF)–like domain hydroxylases, hypoxia-sensing oxygenases, and JmJ histone demethylases. These enzymes play important physiological roles in protein stabilization and the regulation of transcription and translation (19). In particular, prolyl hydroxylation of hypoxia-inducible factor-1α (HIF-1α) transcription factor is one of the most comprehensively studied mechanisms of dioxygenase-mediated transcriptional control (20). Under conditions of abundant oxygen, HIF-1α is hydroxylated, ubiquitinated by the E3 ligase von Hippel–Lindau (VHL) tumor suppressor protein (20), and targeted for proteasomal degradation. Under anoxic or low-oxygen conditions, however, HIF-1α is stable, promoting context-dependent transcription of hundreds of genes implicated in angiogenesis, metabolism, inflammation, and the maintenance of pluripotency (20, 21). αKG-dependent oxygenases can also directly affect components of the translational machinery, including ribosome and translation termination factors. Examples include 2-oxoglutarate and iron-dependent oxygenase domain containing 1 (OGFOD1) and JmjC4, which, upon hydroxylation of...
ribosomal proteins or eRF1, the eukaryotic translation termination factor, improve translation efficiency and accuracy (19, 20).

αKG-dependent oxygenases are also part of biosynthetic pathways critical for cellular homeostasis. For example, 6-N-trimethyllysine and γ-butyrobetaine hydroxylases catalyze reactions essential for carnitine biosynthesis. This process is indispensable for cellular metabolism as carnitine facilitates mitochondrial entry of activated fatty acids (20). Once in the mitochondria, these fatty acids can be further broken down via β-oxidation to generate acetyl-coenzyme A (CoA), which in turn fuels the TCA cycle. αKG-dependent oxygenases have been further implicated in facilitating nucleotide replenishment. Through a process of sequential hydroxylation reactions, thymine 7-hydroxylase converts thymine to thymine-5-carboxyuracil, a precursor for uracil, ensuring that thymine is effectively recycled into uracil (20). Together, these examples underscore the functional diversity of αKG-dependent oxygenases, affecting nucleic acid, protein, and lipid biology.

**IDH1 and the generation of cytoplasmic NADPH**

In addition to its ability to epigenetically control gene expression through production of αKG, IDH1 generates non-mitochondrial NADPH (reduced form of NADP+) that functions as a reducing equivalent needed for lipid biosynthesis and as antioxidant that protects cells from oxidative stress and radiation damage. Studies using a flox-stop-flox--controlled IDH1<sup>R132H</sup> mutant knockin mouse, which, in the absence of Cre recombination, is homozygously null for IDH1, revealed nonessential roles for IDH1 in normal pre- and postnatal development but critical roles in physiologic amino acid catabolism and in protecting against oxidative DNA damage. When compared to wild-type littersmates, IDH1-deficient mice treated with a sublethal dose of lipopolysaccharide showed enhanced hepatic reactive oxygen species (ROS) accumulation, oxidative DNA damage–induced apoptosis, expression of proinflammatory cytokines, and overall increased lethality (22). Similarly, in a wide spectrum of cell types in vitro, IDH1 deficiency was shown to increase cell sensitivity toward ultraviolet B (UVB) or H<sub>2</sub>O<sub>2</sub> through reduced scavenging of ROS (23). In addition, IDH1-deficient mice, when fed a high-protein diet, had notably lower body weight compared to wild-type control littersmates and, after prolonged fasting, showed decreased blood glucose but elevated blood alanine and glycine levels. Accordingly, gluconeogenesis, ammonia, and urea production were diminished, suggesting that IDH1 deficiency and associated drop in αKG impeded the αKG-dependent transamination of glucogenic amino acids (24). While IDH1 deficiency unexpectedly did not affect lipid content, studies of mice harboring liver and adipose tissue–specific expression of an IDH1 transgene revealed the presence of extensive fat pads characterized by adipocyte hypertrophy, accumulation of lipid droplets, and reduced levels of acyl-CoA and malonyl-CoA, i.e., carbon precursor metabolites required for de novo fatty acid synthesis (25). In further support of IDH1 as a critical regulator of lipid metabolism, a recent study identified the microRNA-181a (mir-182)–IDH1 signaling axis as a critical regulator of lipid metabolism. When ectopically expressed, mir-181a, through downregulation of IDH1 mRNA level, decreased the expression of genes implicated in lipid biosynthesis, possibly through αKG-dependent epigenetic effect, while increasing the expression of enzymes involved in β-oxidation. Consistent with these findings, transgenic expression of mir-181a in mice resulted in lower body weight, when compared to wild-type littersmates, and decreased lipid accumulation (26).

**IDH2 and the regulation of mitochondrial homeostasis**

Similar to IDH1, IDH2 is a NADP<sup>+</sup>-dependent homodimer, which catalyzes the reversible oxidative decarboxylation of ICT to αKG, NADPH, and CO<sub>2</sub>. Unlike IDH1, however, IDH2 is a TCA enzyme, which localizes to the mitochondrial matrix (27). While IDH1 shows most prominent expression in the liver with only moderate to absent expression in other tissues, IDH2 is highly expressed in the heart, muscle, and activated lymphocytes (27). By providing mitochondrial NADPH for NADPH-dependent antioxidant enzymes, such as glutathione reductase and thioredoxin reductase, IDH2 maintains a pool of reduced glutathione and peroxiredoxin (28). These molecules, in turn, protect mitochondria from ROS-mediated oxidative damage, ensuing lipid peroxidation and DNA damage (28), and from stress induced by heat shock, cadmium, excess fructose, or tumor necrosis factor-α (TNF-α) (27). Consistent with subcellular localization, tissue expression profile, and molecular mechanism of IDH2, IDH2 knockout mice present with severe mitochondrial dysfunction, in particular loss of mitochondrial redox homeostasis, and exacerbated rates of heart failure associated with cardiac hypertrophy and cardiomyocyte apoptosis (29). Further supporting critical roles of IDH2 in maintaining mitochondrial redox balance, IDH2 deficiency results in increased liver susceptibility to ischemia-reperfusion injury and enhanced oxidative damage to kidney tubule cells (29). Similar to IDH1, the conversion of ICT to αKG catalyzed by IDH2 is reversible. Under conditions of hypoxia or excess glutamate, glutamate dehydrogenases convert glutamate to αKG, which, through IDH2-catalyzed oxidative decarboxylation, is converted to ICT at the expense of NADPH (30). ICT can be converted back to αKG by mitochondrial IDH3. This reaction results in the net production of NADH, which is used in oxidative phosphorylation for ATP production. Alternatively, ICT is isomerized to citrate, which, upon conversion to acetyl-CoA by citrate synthase, fuels lipid biosynthesis (30). αKG is not membrane permeable and does not freely diffuse from mitochondria to the cytoplasm. Instead, αKG and other TCA metabolites are shuttled between mitochondria and cytosol via membrane transporters, which localize to mitochondrial membranes, including the malate shuttle and the citrate transporter protein (CITP). Together, these data show that IDH2 is critical for cellular stress responses, mitochondrial bioenergetics, and macromolecular synthesis to support cell survival and growth.

**IDH3 and mitochondrial bioenergetics**

IDH3 catalyzes the first oxidative reaction of the TCA cycle, resulting in the generation of NADH. Electrons from NADH are passed to oxygen through a series of enzymatic reactions, collectively known as the electron transport chain, which occur within the inner mitochondrial membrane. The flow of electrons from donors, such as NADH, to oxygen as an electron acceptor is an energy-producing exergonic process, which is coupled to the energy-consuming endergonic synthesis of ATP. In addition to the production of energy, the TCA cycle is a biosynthetic hub, which provides metabolites to serve as building blocks for synthesis of macromolecules, including fatty acids, nucleotides, hemes, and porphyrins (3).

As detailed above, αKG produced by IDH3 regulates gene expression through effect on αKG-dependent dioxygenases. Consistent with its impact on gene expression and an evolutionary conserved monopartite nuclear localization signal at amino acid position 124 of the human IDH3α polypeptide, IDH3α can localize to the cell nucleus (31, 32). In particular, Nagaraj et al. (32) demonstrate that multiple
TCA enzymes, including IDH3, localize to the cell nucleus while maintaining a mitochondrial pool during embryonic development. Enzymes associated with the cell nucleus are enzymatically active and, as part of the first half of the TCA cycle, contribute to metabolites, such as acetyl-CoA and αKG. These metabolites are essential for epigenetic control of gene expression and activate the zygotic genome during preimplantation development (32) as well as promote tumorigenesis (31). The differential compartmentalization of specific TCA cycle enzymes, in particular IDH3, suggests that TCA cycle turnover can be fine-tuned by context-specific environmental and developmental cues to regulate energy production, macromolecular synthesis, and gene expression.

**THE BIOLOGY OF CANCER-ASSOCIATED IDH MUTATIONS**

**Prevalence of IDH mutation in cancer**

IDH1 and IDH2 are mutated at high frequency in a spectrum of human cancers. Mutation at Arg132 of IDH1, and at the analogous codon Arg172 of IDH2, represents early initiating events that drive the evolution of low-grade glioma, including grade II to III astrocytoma, oligodendroglioma, and oligodendroglioma associated with Li-Fraumeni syndrome (Fig. 2) (33, 34). These mutations are also detected in grade IV glioblastoma (GBM), referred to as IDH1 mutant GBM, which account for ~10% of all grade IV clinical cases but are absent in pediatric high-grade malignancies and in nongliial subtypes of brain tumors (33). In addition to brain cancers, somatic IDH1 mutations frequently occur in AML and less frequently in MDS and MPNs (6). IDH1 mutations also contribute to the pathogenesis of skeletal disorders, including Ollier disease, defined by multiple central cartilaginous tumors, and Maffucci syndrome, which is characterized by soft tissue spindle cell hemangiomas accompanying cartilaginous tumors (35). IDH1 mutations also define central chondrosarcoma, central and peristeal chondromas, and intracranial chondrosarcoma and have been identified in angioimmunoblastic T cell lymphoma, cholangiocarcinoma, and thyroid carcinomas and as a hallmark of a unique subtype of breast cancer known as solid papillary carcinoma with reverse polarity (Fig. 2) (6). Mutations of IDH genes in the aforementioned cancers are predominantly heterozygous (6).

The prognostic significance of IDH mutations is not well defined. Patients diagnosed with IDH1 mutant GBM have a more favorable prognosis as compared to patients with IDH1 wild-type GBM (36). It remains unclear, however, whether this difference is due to distinct metabolic and genetic rewiring caused by the presence of mutant versus wild-type enzyme or a reflection of a fundamentally different spectrum of co-occurring genetic aberrations that drive distinctive disease pathology. Similarly, in AML, IDH2Arg140 mutation can be associated with better patient prognosis (6), but its prognostic impact may be dependent on co-occurring mutations. For example, AML patients harboring the same IDH2Arg140 mutation in conjunction with a mutation in nucloephin1 (NMP1) have been identified as having a worse overall survival (37). Other studies, however, did not find association between the overall survival of AML patients and the occurrence of IDH1Arg132, IDH2Arg140, or IDH2Arg172 mutations (38). In contrast, IDH1 mutations have been consistently associated with poor prognosis and the presence of late-stage disease in both MDS and MPN, suggesting that IDH mutation may contribute to or drive the progression of chronic MDS and MPN to leukemia (6).

**Enzymatic properties of mutant IDHs**

Mutations in IDH1 and IDH2 are neomorphic gain-of-function mutations, which affect cofactor binding affinity and conformation of the enzymes’ active center. Arg100 and Arg132 in IDH1 and Arg140 and Arg172 in IDH2 form hydrogen bonds with the α-carboxyl and β-carboxyl groups of ICT. When mutated, the enzymes’ binding affinity to ICT decreases, while affinity to NADPH increases. As a consequence, mutations abrogate the IDH forward reaction (i.e., the oxidative decarboxylation of ICT to αKG) (7) and, due to the ensuing conformational change of the active center, result in the catalysis of a partial reverse reaction, in which αKG is reduced to (R)-2-hydroxylglutarate [(R)-2HG] but not further carboxylated (39). All neomorphic IDH mutant enzymes produce (R)-2HG; their allelic frequency, enzymatic property, and association with overall prognosis, however, are markedly different. The IDH2Arg140 mutation is exclusively found in myeloid malignancies. Reflecting its lower intrinsic enzymatic activity, IDH2Arg140 produces less (R)-2HG (38). As discussed below, ~50% of patients diagnosed with (R)-2HG aciduria, an inborn neurometabolic abnormality resulting in aberrant amino acid metabolism and acid buildup, carry a germline IDH2Arg140 mutation but lack other IDH mutations. This suggests that the lower (R)-2HG levels produced by IDH2Arg140 do not adversely affect embryogenesis and perinatal development; other germline IDH mutations, however, due to enhanced generation of pathogenic (R)-2HG, are lethal in utero. Similarly, while Arg132 in IDH1 and Arg172 in IDH2 are structurally analogous residues important for αKG substrate binding, the cytoplasmic and peroxisomal IDH1Arg132 mutant enzyme produces less (R)-2HG compared to mitochondrial IDH2Arg172. Targeting of mutant IDH1 to mitochondria results in enhanced (R)-2HG production, suggesting that IDH1 and IDH2 mutant enzymes have similar intrinsic enzymatic activity and that their activity is regulated through subcellular localization. Mitochondrial levels of αKG are maintained through a variety of anaplerotic reactions and through a high NADPH/NADP+ ratio, which is controlled by interconversion of NADH to NADPH by mitochondrial transhydrogenase (40). Less abundant αKG levels in the cytosol result in lower (R)-2HG production by IDH1Arg132. Consistent with this model, the activity of mutant IDH1 depends on heterodimerization with and increased metabolic flux through wild-type IDH1 to increase local αKG substrate level (39). This functional interplay between mutant and wild-type IDH1 enzymes also suggests that targeting the remaining wild-type IDH1 allele to reduce local αKG availability may represent an additional therapeutic strategy to mitigate protumor effects of IDH1 mutation. Loss of the remaining wild-type IDH1 allele in low-grade glioma is associated with significantly lower levels of (R)-2HG compared to heterozygous IDH1 mutant tumors (36). In addition, due to essential roles of IDH1 and IDH2 in producing cytoplasmic and mitochondrial NADPH, tumor cell survival may also be dependent on basal IDH1/2 activity to maintain cytoplasmic and mitochondrial redox homeostasis.

**Mutant IDH enzymes control cellular growth and differentiation**

When expressed in a wide spectrum of primary and immortalized cells in vitro, including astrocytes, fibroblasts, erythroblast leukemia, murine hematopoietic stem, and myeloid progenitor cells, tumor-derived mutant IDH enzymes can stimulate proliferation, antagonize differentiation, and, together with concomitantly expressed oncogenes, promote soft agar colony formation and transformation (6, 39, 41). In
particular, mutant IDH expression in immortalized astrocytes promotes proliferation and colony formation and, when expressed in erythroleukemia cells, induces differentiation and promotes proliferation independently of stimulation with growth factors. Studies in mice harboring conditional IDH1 and IDH2 mutant knockin alleles revealed that mutant IDH enzymes alone are insufficient to transform primary cells in vivo and instead require cooperating genetic events to promote tumorigenesis. Studies using the aforementioned lox-stop-lox-controlled IDH1^R132H allele, when expressed heterozygously and activated by Cre recombinase in either neural progenitor or hematopoietic cells, revealed that IDH1 mutation is insufficient to provoke a cancer phenotype. IDH1^R132H expression in neural progenitor cells (NPCs) results in extensive cerebral hemorrhage and perinatal lethality. On molecular levels, high-level accumulation of \((R)-2HG\) inhibits prolyl-hydroxylation and subsequent maturation of collagen. Immature collagens accumulate, resulting in an aberrantly formed basement membrane and the initiation of an endoplasmic reticulum (ER) stress response \((42)\). When conditionally expressed in neural progenitors of the subventricular zone, IDH1^R132H promotes the proliferation and invasive properties of stem and transit-amplifying/progenitor cell populations by enhancing DNA methylation and driving the expression of genes implicated in stem cell expansion. As a result, mice developed hydrocephalus and grossly dilated lateral ventricles. It is conceivable that the uncontrolled expansion and spreading of stem/progenitor cells may represent the earliest phase of gliomagenesis \((43)\). When heterozygously expressed in human neural stem cells (NSCs) modified for p53 and ATRX loss, IDH1 mutation promotes low-grade glioma formation in mice. On molecular levels, NSC differentiation was blocked as a result of transcriptional silencing of Sox2 \((44)\). Similarly, virally induced transgenic expression of IDH1^R132H cooperated with platelet-derived growth factor A (PDGFA) expression and loss of inhibitor of cyclin-dependent kinase 4 (CDK4)/alternate reading frame (Ink4a/Arf), α-thalassemia/mentaland retardation syndrome X-linked (Atrx), and phosphatase and tensin homolog (Pten) tumor suppressors to promote GBM formation \((45)\). When expressed in hematopoietic progenitor cells, IDH1^R132H induces a global hypermethylation phenotype and selectively triggers, by increasing symmetric cell division without affecting self-renewal and differentiation capabilities, the expansion of hematopoietic stem and early progenitor compartments but not committed myeloid and lymphoid progenitor compartments. As a result, mice develop splenomegaly and anemia with extramedullary hematopoiesis \((46)\). Studies using a genetically engineered AML mouse model with conditional monoallelic expression of IDH2^R140Q indicated that IDH2 mutation cooperate with HoxA9 and Meis1a overexpression and with mutations in FMS-like tyrosine kinase 3 (FLT3) to initiate and maintain acute leukemia in vivo \((47)\).

The impact of conditional expression of mutant IDHs on tumor progression has also been confirmed in other murine cancer models, which include models of enchondromas, T cell acute lymphoblastic leukemia, and angioimmunoblastic T cell lymphoma \((48)\).

\((R)-2HG\) is an oncometabolite

Because of a chiral center at its second carbon atom, \(2HG\) exists as two enantiomers, i.e., \((R)-2HG\) and \((S)-2HG\). Both enantiomers are considered metabolic by-products, which in IDH wild-type cells are generated by hydroxyacid-oxoacid transhydrogenase and malate dehydrogenase \((49)\). Intracellular concentrations of \(2HG\) are typically maintained in the micromolar range by \(2HG\) dehydrogenase \((2HGDH)\), which convert \(2HG\) to αKG \((49)\). Expression of IDH mutant enzymes, which exclusively generate the \((R)\)-enantiomer, results in elevated concentrations of \((R)-2HG\) which have been hypothesized to contribute to tumorigenesis.

**Fig. 2. Deregulation of IDH enzymatic activity is associated with human disease.** Upward or downward pointing arrows indicate overexpression or downregulation of wild-type IDHs (shown in blue), respectively.
(R)-2HG levels, which can range from 1 mM to as high as 30 mM in cells and exceeds 3 mM within a 2-cm radius from the center of an IDH1 mutant glioma (50). Proton magnetic resonance spectroscopy (MRS) represents a promising advancement in imaging technology for noninvasive assessment of glioma progression. MRS is presently used in preclinical and clinical research and enables the quantification of glioma-associated (R)-2HG levels at concentrations of >1 mM (51). High intracellular accumulation of (R)-2HG suggests that the rate of (R)-2HG production by mutant IDHs exceeds the rate of (R)-2HG oxidation by 2HGDH. Mutant IDH effect on cellular growth and differentiation can be mimicked by continuous addition of cell-permeable (R)-2HG, indicating that sustained provision of high intracellular concentrations of (R)-2HG is necessary and sufficient to mediate oncocgenic effect of mutant IDHs [41, 43].

Central to (R)-2HG oncocgenic effect is its ability to competitively inhibit αKG-dependent dioxygenases, discussed above, to reprogram gene expression associated with cellular growth and differentiation. (R)-2HG—targeted enzymes include TET2, which epigenetically controls gene expression through DNA demethylation, the JmjC family of histone lysine demethylases, and Egfln prolyl-4-hydroxylases, which are critical regulators of HIF activity [13, 52]. IDH mutant tumors through accumulation of (R)-2HG progressively accumulate irreversible changes in the DNA and histone methylation landscape [53]. Recent studies provided additional insight into functional consequences of altered epigenetic states associated with IDH mutation, including the impact of mutant IDH-driven hypermethylation on chromatin topology. IDH mutant gliomas showed extensive hypermethylation at CCCTC-binding factor (CTCF)—binding sites, DNA skeleton sequences that block the interaction between enhancers and promoters. Hypermethylation reduces CTCF binding to DNA, impedes insulator function, and causes a constitutive enhancer to interact aberrantly with PDGF receptor α, a receptor tyrosine kinase and prominent glioma oncogene [54]. It is likely that hypermethylation of CTCF-binding sites through impact on chromatin topography modulates the expression of other genes implicated in (brain) cancer pathogenesis.

More recent studies unraveled a multifaceted activity profile of (R)-2HG, which extends beyond the modulation of dioxygenase activity. Mutual exclusivity of IDH1 and TET2 mutation in certain cancer types, particularly in AML, led to the widely accepted paradigm that mutant IDH1 modulates tumorigenesis through effect on TET2 activity to alter the tumor epigenetic landscape [55]. The lack of complete phenotypic overlap between IDH1 mutant and TET2 mutant cancers, however, suggests that IDH1 can modulate leukemogenesis independently of TET2. In AML, mutations in the Wilms’ tumor suppressor 1 (WT1), a zinc finger–containing transcription factor, are mutually exclusive from TET2 and IDH1/IDH2 mutations. WT1 mutant AML showed a global reduction in 5hmC levels comparable to levels observed in TET2 and IDH1/2 mutant AML. On molecular levels, WT1 via interaction with TET2 and TET3 regulates 5hmC abundance [56]. Analysis of mice harboring IDH1 mutant expression within their hematopoietic system, together with analysis of AML patient tumors, demonstrated that mutant IDH1 down-regulates ataxia telangiectasia mutated (ATM) through effect on histone methylation, resulting in impaired DNA repair, increased sensitivity to DNA damage, and reduced hematopoietic stem cell renewal [57]. Similarly, in glioma cells and tumors, IDH1 mutation through production of (R)-2HG inhibits ALKBH DNA repair enzyme and, in so doing, reduces DNA repair kinetics, increases DNA damage, and sensitizes glioma cells to alkylating agents [21]. In addition to affecting enzymes implicated in DNA repair, (R)-2HG also regulates a variety of different metabolic enzymes to rewire tumor cell metabolism. In GBM, (R)-2HG inhibits the αKG-dependent branched-chain amino acid transaminase 1 (BCAT1) and BCAT2, which catalyze the reversible transamination of branched-chain α-keto acids to the branched-chain amino acids valine, leucine, and isoleucine. These amino acids are essential for cellular growth, and mutations in BCAT1 and BCAT2 result in autosomal recessive disorders, termed hypervalinemia and hyperleucine-iso leucinemia. Mutant IDH1-derived (R)-2HG can inhibit BCAT activity and, in so doing, decreases glutamate levels and increases glioma cell dependency on glutaminase for the synthesis of glutamate and glutathione. As a result, IDH1R132H-expressing glioma cells and derivative orthotopic tumors are uniquely sensitive to pharmacological inhibition of glutaminase [58]. In addition to competitive inhibition of BCAT enzymatic activity by (R)-2HG, IDH1 mutation down-regulates BCAT expression through epigenetic effects [58], demonstrating that IDH1 mutant gliomas regulate BCAT activity through enzyme inhibition and epigenetic control of expression. Similar to glioma, AML tumors with wild-type IDH1 are characterized by increased branched-chain amino acid pathway activity, including high BCAT1 expression and activity. BCAT1 knockdown causes accumulation of αKG, which, through activation of EGLN1, triggers HIF-1α protein degradation and growth arrest. Similar to leukemia cells carrying a mutant IDH1, expression of BCAT1 in IDH1 wild-type AML cells decreases intracellular αKG levels and causes TET enzyme–dependent DNA hypermethylation [59].

In addition to the epigenetic control of gene expression, (R)-2HG can regulate transcript stability. By inhibiting FTO activity, (R)-2HG increases global N6-methylenadenosine (m6A) RNA modification. Leukemia cells treated with cell-permeable (R)-2HG or engineered to express mutant IDH1 showed decreased m6A stability of cellular Myelocytomatosis (cMYC) and CCAAT enhancer-binding protein alpha (CEBPA), suggesting that (R)-2HG, while promoting tumor initiation, can antagonize later-stage progression of blood cancers [60]. Furthermore, (R)-2HG can directly impair mitochondrial energy metabolism by inhibiting complex IV (cytochrome c oxidase) and complex V (ATP synthase) of the electron transport chain [13, 53]. The ensuing accumulation of excess electrons when transferred to oxygen results in the generation of superoxide species, which can promote cellular transformation [13]. Through competitive inhibition of the TCA cycle enzyme succinate dehydrogenase (SDH), (R)-2HG accumulation can also lead to accumulation of succinyl-CoA, which results in hypersuccinylination. Consistent with this model, hypersuccinylination inhibits mitochondrial respiratory function, causing mitochondrial membrane hyperpolarization and induction of B cell lymphoma 2 (Bcl-2) protein expression, which, in turn, renders hypersuccinylated cancer cells apoptosis resistant [61]. Up-regulation of Bcl-2 can be exploited by treating IDH1 mutant cells with Bcl-2 inhibitors, as detailed below and summarized in Table 1.

2HG-mediated metabolic neurodegeneration—Pathogenic effect of IDH mutation beyond cancer

2HG aciduria (2HGA) is a rare inherited metabolic disorder characterized by a wide spectrum of clinical presentation [62]. Stereoisomeric property of 2HG gives rise to three distinct types of aciduria, i.e., (S)-2HG aciduria (S2HGA, type I), (R)-2HG aciduria (R2HGA, type II), and combined S2HGA/R2HGA (type III). S2HGA (type I) and R2HGA (type II) are biochemically distinct disease entities with unique genetic and phenotypic predispositions. Both S2HGA and
R2HGA are predominantly caused by specific mutations in enantiomer-specific, mitochondrial-localized 2HG dehydrogenases, i.e., (S)-2HG dehydrogenase (S2HGDH) and (R)-2HG dehydrogenase (R2HGDH), respectively (62), which oxidize 2HG to αKG (49). Each type of 2HGA has common and unique symptoms and pathologies. S2HGA (type I) is the most common form of 2HGA. Elevated levels of (S)-2HG in the urine, plasma, and cerebral spinal fluid were found to be associated with psychomotor retardation, dystrophy, delayed skeletal age, and progressive central nervous system (CNS) deterioration (62). As a result of neuronal degeneration, patients invariably present with encephalitis, seizures, abnormal extrapyramidal motor function, and intellectual impairment (62). While S2HGDH autosomal recessive mutations represent the underlying cause of nearly all reported cases of S2HGA, R2HGA (type II) exhibits a more complex genetic etiology. Recessive D2HGDH mutation accounts for roughly half of all R2HGA cases, while autosomal dominant, heterozygous germline mutations in IDH2 characterize the remaining R2HGA cases (Fig. 2) (63). R2HGA cases defined by IDH2 mutation exhibit a worse prognosis with more severe symptoms, including ataxia (53, 63). IDH1 mutations were not observed in any patients with R2HGA (63). Combined S2HGA/R2HGA (type III) is characterized by elevated levels of both (S)-2HG and (R)-2HG. The disease manifests in early infancy, presenting with severe seizures, encephalopathy, hypotonia, feeding problems, and impaired breathing. Patients typically succumb to the disease before the onset of early childhood (62). Recently, recessive mutation in SLC25A1, the mitochondrial citrate carrier, was identified as the cause of type III 2HGA (62). Inability to import citrate into the mitochondria impairs IDH2 and IDH3 function, likely resulting in the buildup of cytotoxic ROS (62).

**IDH3 mutation, neurological impairment, and retinal degeneration**

Mutations in IDH3A have been identified in individuals with inherited retinal diseases (IRDs), which represent a major cause of incurable blindness in children and young adults, and are characterized by progressive degeneration of photoreceptor and/or retinal pigment epithelium cells. Retinitis pigmentosa (RP) is the most common IRD, affecting 1 in 4000 individuals worldwide (64). Patients diagnosed with typical RP suffer from night blindness and visual field constriction, with progressively decreasing visual acuity (64). Whole-genome sequencing revealed a spectrum of IDH3 mutation associated with RP (Fig. 2). In particular, individuals with homozygous missense mutation in codon 304 of IDH3A showed the most severe phenotype and suffer from infantile encephalopathy with peripheral and
autonomic nervous system involvement and RP (65). Pierrache et al. (66) identified a series of additional IDH3A variants in patients diagnosed with typical autosomal recessive RP (arRP) and with arRP and macular pseudocoloboma. Symptom severity was variable, ranging from night blindness to severe visual impairment. It is conceivable that some missense variants might be hypomorphic variants that allow residual IDH3 activity. All patients with arRP and macular pseudocoloboma carried a null variant, which could explain the more severe phenotype (66). When mutations in IDH3C as underly ing cause or contributor to neurological defect remain elusive, biallelic variants in IDH3B were also found to be associated with typical arRP, but not with arRP with macular pseudocoloboma or systemic neurological impairment (67). Association of IDH3 compromise with IRDs is not surprising, as retina cells have a particularly high-energy requirement, with their mitochondria operating at 70 to 80% of their maximal rate. Mitochondrial reserve capacity is limited, and energy metabolism is highly dependent on TCA cycle activity. Consequently, retina cells are highly sensitive to mitochondrial dysfunction as a result of IDH3 compromise (66, 67).

THE EMERGING ROLE OF WILD-TYPE IDH3 IN CANCER
Wild-type IDH1, GBM progression, and therapy susceptibility
As detailed above, oncogenic mutations in IDH1 have been reported for lower-grade gliomas and mutant IDH1 GBM, which represent approximately 10% of all clinical grade IV cases. Approximately 90% of all grade IV glioma, however, do not harbor this mutation, and they are classified as wild-type IDH1 GBM (68). Using bioinformatics, targeted metabolomics, and a series of murine orthotopic gain- and loss-of- function models, recent reports demonstrated that up-regulation of IDH1 and associated increase in cytoplasmic NADPH and αKG are novel mechanisms of metabolic reprogramming in wild-type IDH1 GBM (Fig. 2) (69). Wild-type IDH1 antagonized ROS-mediated cell death, caused resistance toward epidermal growth factor receptor (EGFR)–targeted therapy (69) as well as radiation treatment (70), and promoted a more dedifferentiated cell state through effect on histone demethylases and epigenetic gene regulation (69). In addition, NADPH is a critical and possibly rate-limiting cofactor required for fat and cholesterol biosynthesis (25). Consequently, cancer-associated wild-type IDH1 through enhanced NADPH production promotes lipid biosynthesis (69). Cancer cells require lipids, in particular fatty acids, for membrane proliferation and composition, energy storage, and the generation of signaling molecules. Inhibition of lipogenic enzymes leads to an increase in the level of polyunsaturation, rendering cells more susceptible to oxidative stress—induced cell death and impairing the deformability and lateral fluidity of cell membranes. The major transcriptional regulator of fatty acid metabolism is the sterol regulatory element binding protein 1 (SREBP-1), a transcription factor that induces the expression of genes implicated in de novo lipid synthesis, including IDH1 (71).

The emerging role of IDH3 in cancer
The impact of IDH3 on cellular transformation and tumor progression is context dependent. Down-regulation of IDH3α in the tumor stroma promotes the transformation of fibroblasts into cancer-associated fibroblasts by up-regulating HIF-1α and inducing a switch from oxidative phosphorylation to glycolysis (72). In cervical epithelial adenocarcinoma cells, on the other hand, down-regulation of IDH3α leads to HIF-1α inactivation and inhibition of tumor progression (21). Additional studies have demonstrated IDH3α levels to correlate with poor survival of lung and breast cancer patients, indicative of tumor-promoting properties of IDH3α (21). In GBM, IDH3α protein is up-regulated compared to normal brain tissue, in particular in tumor cells located at the leading edge and within cells of the tumor-associated but not normal brain endothelium (Fig. 2) (31). Reflecting its central role in regulating TCA cycle activity and cellular respiration, IDH3α ablation reduces carbon flux through the TCA cycle, shunts energy metabolism, induces a shift toward increased glycolysis and pentose phosphate shunt usage, and robustly reduces the tumorigenic potential of transformed astrocytes and patient-derived glioma-initiating cells (31). In cultured glioma cells and patient-derived tumor specimens, IDH3α showed cell cycle–dependent extramitochondrial localization, with S phase–arrested cells demonstrating predominant accumulation of IDH3α in cytosol and at the nuclear periphery. Here, IDH3α colocalized and interacted with cytosolic serine hydroxymethyltransferase (cSHMT), a rate-limiting enzyme of the de novo thymidylate synthesis pathway (73). cSHMT appropriates folate species between nucleotide synthesis and methionine salvage pathways (73). Loss of IDH3α expression resulted in cSHMT compromise, which, in turn, reduced rates of thymidylate synthesis, while carbon flux into the salvage methionine cycle increased, resulting in elevated levels of the methyl donor S-adenosyl methionine and DNA methylation (31).

IDH3A AND THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES
IDH enzymes have been implicated in neurodegeneration due to their role in cellular metabolism and redox homeostasis (Fig. 2). Cytosolic and mitochondrial NADPH supplied by IDH1 and IDH2 is critical for the regeneration of glutathione that protects neuronal cells from oxidative damage (74). Glutathione impairment has also been implicated in the pathogenesis of PD, AD, HD, ALS, and Friedreich’s ataxia (75). As a result of decreased levels of reduced glutathione, oxidative damage to lipids, proteins, and nucleic acids is increased in postmortem brain tissue of PD patients (74). Similarly, decreased levels of glutathione have been observed in AD and HD (75); a direct link with IDH dysregulation, however, has yet to be elucidated.

In PD, IDH dysregulation has been directly linked to protein de- glycose DJ-1. DJ-1 emerged as a critical redox-sensitive chaperon and oxidative stress sensor, which inhibits the aggregation of α-synuclein while transcriptionally controlling IDH expression under oxidative stress through activation of the Kelch-like ECH-associating protein 1 (Keap1)–nuclear factor E2–related factor 2 (Nrf2) pathway (76). Mirroring the DJ-1 loss-of-function phenotype, drosophila lacking IDH expression showed lower levels of NADPH, increased cellular ROS, dopaminergic neuron degeneration, and loss of locomotive function (76). Notably, in drosophila, all IDH paralogs are expressed from a single IDH gene. To assess the contribution of IDH1 versus IDH2 to disease phenotypes, DNA complementation experiments in mammalian DJ-1 null cells using IDH1 and IDH2 constructs revealed that both paralogs can rescue ROS-induced cell death triggered by DJ-1 loss (76). Further functional experiments using the yeast Saccharomyces cerevisiae and a murine neurotoxin model of PD confirmed critical roles for IDH1 in antagonizing ROS accumulation and overall cell toxicity in response to α-synuclein expression (77) and identified IDH2 as a cytoprotective factor in dopaminergic neurons (78). In addition to the regulation of neuronal redox homeostasis,
deregulated IDH expression drives or contributes to extensive metabolic rewiring in pathogenic neurons from ALS, PD, and HD patients. Impairment of oxidative phosphorylation is a hallmark of ALS pathogenesis. To compensate for reduced oxidative phosphorylation, ALS mutant motor neurons (bearing the SOD1<sup>G93A</sup> mutation) increase metabolic flux through other pathways, including fatty acid oxidation and TCA cycle (79). Heightened pathway activity is associated with increased expression of enzymes implicated in β-oxidation and TCA cycle turnover, in particular IDH3 (79). Up-regulation of IDH3 in ALS motor neurons further facilitates the metabolic shift toward fatty acid utilization to meet cellular energy requirements (79). Deregulation of IDH3 expression in neurodegeneration further reflects important function of this enzyme in neuronal function. Homozygous mutation of IDH3A was associated with severe epileptic encephalopathy in infants (65). In addition, a recent study linked IDH3α and mitochondrial metabolism to synaptic transmission (80). Loss of IDH3α function and the ensuing drop in αKG level impaired synaptic transmission in photoreceptors and larval neuromuscular junctions of drosophila flies. Flies with IDH3α loss of function and reduced αKG levels showed abnormal synaptic transmission, which could be restored by supplying flies with αKG through their diet. On molecular levels, αKG affected the dynamics of the Ca<sup>2+</sup> sensor synaptotagmin1 (Syt1), which, upon Ca<sup>2+</sup> binding, initiated synaptic vesicle (SV) fusion with the plasma membrane. αKG directly enhanced Syt1-lipid interaction, caused a robust increase in fusion between vesicles, and antagonized synaptic transmission defect caused by IDH3α loss (80).

### FROM BENCH TO BEDSIDE: SMALL-MOLECULE INHIBITORS OF MUTANT IDH1 AND IDH2

Mutations in IDHs characterize a broad spectrum of solid and systemic cancers with starkly different phenotypic and genotypic presentation. In addition, IDH1 and IDH2 mutations occur at different frequencies in different tumor types and represent prognostic features in patients diagnosed with some, but not all, tumor types. Consequently, as detailed below, patient responses to mutant IDH inhibitors vary between tumor types, with hematological malignancies being exquisitely sensitive to mutant IDH inhibitors and with gliomas lacking pronounced treatment responses.

#### Mechanism of small-molecule–based mutant IDH inhibitors

A series of compounds have been developed to inhibit mutant IDH1, mutant IDH2, or both (see Table 2 for potency, specificity, cancer indication, and clinical trial identifiers). For many of these compounds, the mechanism of inhibition has been elucidated at the molecular level by defining co-crystal structures of the inhibitor bound to enzymes by x-ray crystallography. Most inhibitors regulate enzyme activity allosterically, rather than competing for substrate binding to the active site. GSK321 and IDH305 inhibit mutant IDH1 by engaging an allosteric pocket formed when the enzyme adopts an open configuration. The inhibitor does not occupy the NADPH-binding site or engage the active center at Arg<sup>132</sup>, but instead one molecule of GSK321 binds to each monomer and induces structural changes in the tertiary structure of the enzyme, resulting in a catalytically inactive conformation (81, 82). The IDH2<sup>R140Q</sup>-specific inhibitors AGI-6780 and AG-221 bind tightly to the homodimer interface, locking the enzyme in an open, catalytically inactive conformation while at the same time blocking NADPH binding (83, 84). Similarly, AG-881, the first dual-specific inhibitor targeted to both mutant IDH1 and IDH2, binds an allosteric pocket present in both enzymes and locks enzymes in an inactive conformation (85). Another mechanism of inhibition is the direct interaction of a mutant IDH1 inhibitor with Asp<sup>279</sup>, one of the residues that chelate the Mg<sup>2+</sup>/Mn<sup>2+</sup> ion essential for catalysis. The binding of the compound prevents the enzyme from adopting the catalytically competent conformation, which is supported by Mg/Mn<sup>2+</sup> binding (86).

A myriad of preclinical studies characterized the effects of mutant IDH inhibition on cell metabolism, growth, and tumorigenicity and demonstrated that small-molecule inhibition robustly reduces (R)-2HG level (81, 82, 87). Mutant IDH inhibition resulted in enhanced progenitor cell differentiation, which was associated with an increase in differentiation and a decrease in stem cell marker expression, as shown in AML blast cells ex vivo (82) and in glioma-initiating cells in vitro and derivative tumors in vivo (87). When treated with GSK321, IDH1 mutant AML cells showed genome-wide hypomethylation, predominantly within intergenic and intronic regions, mostly at >5 to <500 kb from the nearest transcription start site. Gene set enrichment analysis of loci associated with hypomethylated regions demonstrated that inhibitor treatment most prominently affected cell cycle progression, stem cell fate, and differentiation processes (82).

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**Wild-type IDH1 OE**

**Wild-type IDH1 compromise/ presence of IDH<sup>R152H</sup>**

**Wild-type IDH1 compromise/ presence of IDH<sup>R152H</sup> - high Glu flux**

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**Fig. 3.** Compensation for wild-type IDH1 loss of function in GBM tumors characterized by high glutamate flux to support TCA and lipid biosynthesis activities. AA, amino acids; Ac-CoA, acetyl–coenzyme A; CIT, citrate; Glu, glutamate; GLUD, glutamate dehydrogenase; OE, overexpression; Pyr, pyruvate.
Similarly to the effect of mutant IDH1 inhibitors, treatment of AML cancer stem, progenitor, and transformed cell lines with the mutant IDH2 inhibitor AG-221 reduced growth factor–independent proliferation, antagonized histone and DNA hypermethylation, impaired self-renewal while promoting differentiation, and elicited a dose-dependent increase in overall survival of tumor-bearing mice (84). In contrast to the effect of mutant IDH inhibition in AML, the antitumor effect of mutant IDH inhibitors in glioma was more variable. AGI-5198, a mutant IDH1-specific inhibitor, was administered to patient-derived xenograft (PDX)–bearing mice at high and low dosage. While both dosages effectively inhibited proliferation and equally reduced tumor progression, histone methylation and associated tumor cell differentiation were induced in mice treated with high inhibitor concentration only. These data suggest that the epigenetic control of differentiation is not required for the antitumor effect in response to IDH1 inhibition and that mutant IDH1 regulates glioma cell proliferation and differentiation through distinct pathways characterized by different responsiveness toward (R)-2HG (86). While this initial study pointed to mutant IDH1 as a druggable driver mutation that is required for the evolution of low-grade glioma, subsequent studies using different IDH1 inhibitors in multiple PDX models showed that IDH1 inhibition and depletion of (R)-2HG affect low-grade glioma growth in some, but not all, models (87–89). Assuming that the blood-brain permeability and the inhibitory activity of different compounds are similar, the different treatment responses likely reflect the genetic heterogeneity in the different models used. It is conceivable that different IDH1 mutant glioma cell lines are characterized by distinct secondary driver mutations and may represent more or less advanced stages of glioma malignancy. In more malignant models, proliferation and differentiation block induced by IDH1 inhibition might be insufficient to halt tumor growth in response to strong mitogenic signaling, e.g., initiated by a hyperactivated phosphatidylinositol 3-kinase–Akt signaling pathway. Consistent with this hypothesis, IDH2 mutant AML with co-occurring mutations in the Ras pathway show decreased sensitivity toward mutant IDH2 inhibition (90). It is also conceivable that more advanced gliomas with prolonged exposure to mutant IDH1 expression are less susceptible to reversing epigenetic changes once (R)-2HG levels have been normalized via inhibition of mutant IDH1.

Several inhibitors that were developed to target mutant IDH1 showed significant inhibitory activity against wild-type IDH1 (Table 2). Treatment of glioma-initiating cells harboring high-level expression of wild-type IDH1 with GSK864, a compound structurally

### Table 2. IDH mutant inhibitors.

| Drug   | Target                  | IC₅₀       | Cancer indication                     | Clinical trial number |
|--------|-------------------------|------------|---------------------------------------|-----------------------|
|        | Mutant      | Wild-type/mutant heterodimer | Wild type |                               |                        |
| AGI-5198 | IDH₁R₁³₂H | 0.07 µM | N/A | >100 µM | Low-grade glioma             | N/A                    |
|         | AGI-5198 | IDH₁R₁³₂H | 12 µM | 12 µM | 71 µM | Low-grade glioma Hematologic malignancies Advanced cholangiocarcinoma | N/A |
| AG-120   | IDH₁R₁³₂H | 12 µM | 12 µM | 71 µM | Low-grade glioma Hematologic malignancies Advanced cholangiocarcinoma | N/A |
| AGI-6780 | IDH₂R₁⁴₀Q | 170 µM | 120 µM | 2.7 µM | Hematologic malignancies | N/A |
| AG-221   | IDH₂R₁⁴₀Q | 0.32 µM | 0.31 µM | 39.8 µM | Hematologic malignancies | N/A |
| AG-881   | Pan-specific mutant IDH | 31.9 nM (IDH₁R₁³₂H) | 31.7 nM (IDH₂R₁⁴₀Q) | N/A | N/A | Hematological malignancies Low-grade glioma Advanced solid tumors | N/A |
| BAY1436032 | IDH₁R₁³₂H | 15 nM | N/A | 20 µM | Advanced malignancies | N/A |
| GSK321   | IDH₁R₁³₂H | 4.6 nM | N/A | 46 nM | N/A | Advanced malignancies | N/A |
| GSK864   | IDH₁R₁³₂H | 15.2 nM | N/A | 46.6 nM | N/A | Advanced malignancies | N/A |
| IDH305   | IDH₁R₁³₂H | 0.027 nM | N/A | 6.14 µM | Hematological malignancies | N/A |
| MRK-A    | IDH₁R₁³₂H | 5 nM | N/A | N/D | N/A | Advanced malignancies | N/A |

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related to GSK321, resulted in a dose-dependent decrease of the NADPH/NADP⁺ ratio, while cells that have low IDH1 expression, including nontransformed cortical astrocytes, did not respond to treatment (69). Moreover, when tested in a panel of transformed glioma cells and patient-derived tumor spheres, GSK864 reduced cell viability and stem cell frequency, increased apoptosis when used in combination with inhibitors of receptor tyrosine kinases, and delayed tumor progression in vivo (69).

Most of the aforementioned compounds have been evaluated in phase 1 clinical trials for the treatment of IDH mutant AML, MDS, and glioma. All compounds tested had favorable safety profiles, and in dose-escalation studies, a maximum tolerated dose was not reached. Compound administration resulted in reduction of plasma (R)-2HG levels in cancer patients and was associated with tumor responses. IDH305 and AG-120 treatment in patients diagnosed with hematologic malignancies resulted in complete disease remission in 9.5 and 19% of patients and partial responses in 19 and 41% of patients, respectively (91, 92). Results from a recent phase 1 dose-escalation and dose-expansion study of AG-120 in AML patients harboring mutant IDH1 revealed a significant correlation between lower somatotubulin burden and complete remission or complete remission with partial hematologic recovery. While single-gene mutations were not predictive of clinical response or AG-120 treatment resistance, mutations in tyrosine kinase pathway components, including mutations in NRAS and FLT3 and less frequently KRAS and PTPN11 mutations, were enriched in nonresponding patients with relapsed or refractory AML (92). Similarly, the first-in-human phase 1/2 study of the IDH2 mutant inhibitor AG-221 in patient with myeloid malignancies demonstrated that the treatment was generally well tolerated. (R)-2HG levels were reduced by the second cycle of treatment, and the overall response rate was 40.3%, with 19.3% of patients reaching complete remission (93). As reported in a 2016 phase 1 study, administration of AG-221 to patients with MDS resulted in a 50% response, with only two patients experiencing disease progression (94). The U.S. Food and Drug Administration (FDA) approved AG-221 in August 2017 and AG-120 in July 2018 for adult patients with IDH mutant relapsed or refractory AML. Recent clinical studies in patients with mutant IDH1 relapsed/refractory AML revealed that AG-120 was well tolerated, with an overall response rate of 41.9% (95). AG-120 was also tested in patients with previously untreated IDH mutant AML. Similar to previous clinical studies, AG-120 was well tolerated and induced durable remission and progression independence (96). DiNardo et al. (97) showed a strong correlation between (R)-2HG levels and overall survival in AML. Specifically, the authors found that serum (R)-2HG levels can predict both IDH mutational status and patient prognosis; IDH mutant patients with serum levels of <200 ng/ml have shorter overall survival compared to patients with serum (R)-2HG of ≥200 ng/ml (97). A supporting article by Wang et al. (98) demonstrated that increased (R)-2HG serum level is a predictor of poor prognosis and could potentially serve as a marker for AML patient outcome. Correspondingly, patient response to mutant IDH inhibitors correlated with patient prognosis. Stein et al. (99) presented results from an AML clinical study using the mutant IDH2 inhibitor AG-221. The authors noted that the extent of (R)-2HG reduction elicited by the treatment correlated with patient outcome. Last, phase 1 dose-escalation studies with the pan-specific mutant IDH inhibitor AG-881 in patients with recurrent/progressive mutant IDH1/2 glioma and nonglioma solid tumors revealed a favorable safety profile at doses below 100 mg daily (100).

Combination therapies with chemotherapy and targeted therapy
Mutant IDH inhibition and associated decrease in tumor-derived (R)-2HG reduces the tumorigenic potential of leukemic blast and glioma-initiating cells in vitro and in murine cancer models in vivo. While FDA-approved small-molecule inhibitors targeted to mutant IDHs elicit partial responses or complete remission in AML patients, preclinical responses in low-grade glioma are less consistent and robust. In addition, AML patients with mutant IDH2, while displaying initial responses to AG-221, can acquire clinical resistance toward the inhibitor and develop recurrent disease associated with an increase in (R)-2HG. Therapy resistance is driven by the emergence of second-site IDH2 mutations, which can occur in either cis or trans, and affects residues that are located at the dimer interface recognized by the inhibitor (101). In addition to second-site mutations, IDH isomerase switching from cytoplasmic mutant IDH1 to mitochondrial mutant IDH2 or vice versa can also contribute to the development of resistance (102). These studies point to selective pressure to maintain (R)-2HG production in IDH mutant cancers and suggest that recurrent tumors expressing both mutant IDH1 and IDH2 should be sensitive to treatment using dual-specific mutant IDH1/2 inhibitors or to cotreatment with mutant IDH1- and IDH2-specific small molecules. Notably, IDH mutant disease treated with dual-specific inhibitors, such as AG-881, can result in disease progression propelled by (R)-2HG–independent mechanisms, suggesting that alternative genetic mechanisms can compensate for (R)-2HG to drive tumor progression (102).

To enhance the antitumor effect of IDH mutant inhibitors, a series of preclinical studies investigated synthetic lethal interactions of mutant IDH compromise, in particular in low-grade gliomas, to inform combinatorial regimens to more effectively antagonize mutant IDH1 tumor progression (Table 1). Most IDH1 mutant gliomas are characterized by O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, which, in turn, results in decreased MGMT expression, reduced MGMT–dependent DNA repair, and increased sensitivity toward DNA-methylating chemotherapy, foremost temozolomide (103). In addition to impaired MGMT–dependent DNA repair, enhanced chemosensitivity of mutant IDH1 tumors is also a result of compromised oxidative metabolism and associated reduction in NAD⁺ level. When comparing the metabolic profiles of IDH1 mutant glioma-initiating cells in the presence or absence of an IDH1 inhibitor, Tateishi et al. (89) found that IDH1 mutant tumors through an (R)-2HG–independent mechanism downregulate the expression of nicotinate phosphoribosyltransferase 1 (Naprt1), the rate-limiting enzyme of the NAD⁺ salvage pathway. As a result, IDH1 mutant tumors are vulnerable to NAD⁺ depletion via concomitant nicotinamide phosphoribosyltransferase (NAMPT) inhibitor (89), and local nanoparticle-mediated delivery of an NAMPT inhibitor (GMX-1778) reduces tumor progression selectively in IDH1 mutant low-grade glioma PDX models (104). The NAD⁺ coenzyme is also a critical cofactor for poly(ADP-ribose) polymerase (PARP)–mediated base excision repair of chemotherapy-induced DNA damage. The combination of temozolomide and NAMPT inhibitor reduced tumor progression in IDH1 mutant gliomas more effectively compared to either monotherapy, independent of MGMT methylation and mismatch repair status (105). Additional tumor sensitivities associated with IDH mutations were identified in MPNs, AML, and intrahepatic cholangiocarcinomas (ICCs), an aggressive tumor of the liver bile duct. In MPNs, gain-of-function
mutations in the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway co-occur with IDH1 and IDH2 mutations, and the combined inhibition of both mutant JAK2 and mutant IDH1 enzymes had more pronounced antileukemic effects than either therapy alone (106). A large-scale RNA interference screen in AML identified Bcl-2 as a gene with synthetic lethality to IDH1R132H. IDH1 mutant AML cells and derivative tumors, when compared to specimens with IDH1 wild-type status, are more sensitive to the Bcl-2 inhibitor ABT-199 due to (R)-2HG inhibition of cytochrome c oxidase, which lowers the threshold for apoptosis induction in response to Bcl-2 inhibition (107). Finally, ICC tumor cells showed heightened sensitivity toward the multit kinase inhibitor dasatinib, which, through specific inhibition of the nonreceptor tyrosine kinase SRC, induced pronounced tumor cell apoptosis and ICC xenograft regression (108).

**IDH mutant status defines the tumor-associated immune system**

Studies using a genetically engineered murine mouse model with virally induced overexpression of either IDH1R132H or IDH1 wild-type transgenes and concomitant p53 knockdown and PDGF expression suggested that IDH1 mutation through (R)-2HG–driven epigenetic effect down-regulates the expression of leukocyte chemotaxis factors (109). In particular, the infiltration of immune cells linked to poor prognosis in many cancer types, such as macrophages, microglia, monocytes, and neutrophils, was dampened in IDH1 mutant gliomas (109), and consistently, the systemic depletion of neutrophils selectively slowed down the disease progression of IDH1 wild-type tumors but had no further effect on the progression of IDH1 mutant tumors (109). Significant infiltration of IDH1 wild-type gliomas with immune cells, such as macrophages, points to unique immune-related vulnerabilities that can be therapeutically explored, e.g., by co-inhibiting wild-type IDH1 enzyme activity (see discussion below on targeting the wild-type form of IDH1) and macrophage function using macrophage antagonists, such as CSF1R.

In further support of immune-related differences between IDH1 wild-type and mutant gliomas, in silico analysis using The Cancer Genome Atlas (TCGA) dataset found that wild-type glioma showed enrichment of gene signatures associated with tumor-promoting M2 macrophage and neutrophils (68) and high-level expression of immunoregulatory molecules, such as PD-L1, which correlated with poor patient outcome in wild-type IDH1 as compared to mutant gliomas (110). Furthermore, IDH1 mutations affected cytotoxic T lymphocyte (CTL) and natural killer (NK) cell biology. As demonstrated in TCGA clinical low-grade glioma specimens and syngeneic mouse tumors, IDH1 mutation through increased (R)-2HG production diminished intratumoral STAT1 activation, which, in turn, resulted in down-regulation of CTL-attracting chemokines, such as CXCL10, and reduced number of tumor-infiltrating CTLs. Pharmacological inhibition of mutant IDH1 reversed the observed immunosuppressive phenotype and enhanced the antitumor effect of peptide-based vaccine immunotherapy, supporting the notion that IDH1 mutant inhibitors enhance immunotherapy and tumor cell killing in IDH1 mutant glioma patients (111). IDH1 mutant low-grade glioma cells are also resistant toward NK cell–mediated cytotoxicity due to hypermethylation and epigenetic silencing of NK group 2D ligands. Treatment of IDH1 mutant cells with the DNA methyltransferase inhibitor decitabine, by reversing the hypermethylation phenotype, increased glioma cell sensitivity toward NK cell lysis (112).

In addition to (R)-2HG ability to cause tumor cell–intrinsic epigenetic alterations, high abundance of (R)-2HG levels in body fluids of IDH mutant AML and glioma patients also suggested that (R)-2HG may shape the tumor-associated immune system in an autonomous paracrine way (53). (R)-2HG levels are significantly higher in T cells from AML patients with IDH2 mutation compared to healthy controls or AML patients with wild-type IDH2. Although (R)-2HG is cell impermeable and its mode of transport remains ill defined, (R)-2HG is secreted by IDH1 mutant tumor cells and taken up by T cells (53). Tumor-derived (R)-2HG suppressed the proliferation of activated T cells by perturbing nuclear factor of activated T cells (NFAT) and Ca2+ signaling, as well as polyamine biosynthesis (113), and by destabilizing HIF-1α, inducing a shift from glycolysis to oxidative phosphorylation. Collectively, the studies suggest that intratumoral immune suppression in IDH1 mutant tumors may occur through tumor cell–intrinsic effects of (R)-2HG on immune cell recruitment and through paracrine metabolic effect on tumor-infiltrating T cells.

Last, the identification of an immunogenic epitope within mutant IDH1 enabled the development of vaccines using peptides encompassing the mutated region of the IDH1R132H enzyme. When presented on major histocompatibility complex class II, these neoantigens induced mutation-specific CD4+ T helper 1 (Th1) responses and reduced progression of murine syngeneic IDH1R132H gliomas (114). An IDH1 mutation–specific vaccine is currently being evaluated in two clinical trials in patients diagnosed with IDH1 mutant glioma (NCT02193347 and NCT02454643). Because of suppression of the tumor-associated immune system through (R)-2HG–dependent mechanisms described above, antitumor immunity induced by IDH1R132H specific vaccines can likely be improved by cotreatment with IDH1 mutant small-molecule inhibitors to reduce tumor-derived (R)-2HG and with checkpoint inhibitors (53, 113).

**CONCLUSION AND FUTURE DIRECTIONS**

The discovery of neomorphic cancer-associated IDH1 and IDH2 mutation more than 10 years ago (6, 36) fueled the field of cancer metabolism, ignited drug development efforts to pharmacologically target mutant enzymes, and resulted in the FDA approval of two small-molecule inhibitors for the treatment of AML. A comprehensive molecular view of mutant IDH oncogenicity has emerged since then, which pointed to mutant IDH enzymes as critical regulators of redox homeostasis, metabolic flux, and gene expression. Among the many biological phenotypes caused by expression of mutant IDHs, the suppression of the tumor-associated immune system emerged as one of the latest and probably most intriguing examples. The exploration of IDHs will undoubtedly continue to establish IDH mutation as an important event in the genesis and progression of a wide spectrum of systemic and solid cancers and will unravel novel and unexpected functions of nonmutated IDHs in human disease. Below, we discuss some of the most critical questions.

**Does the IDH1 status of progenitor cell populations define distinct GBM disease entities?**

IDH1 wild-type and mutant GBM, while histologically similar, represent distinct disease entities. IDH1 mutant GBMs are predominantly located in frontal lobe structures and maintain a gene signature associated with the proneural subtype of GBM. In contrast, IDH1 wild-type...
tumors show a more widespread anatomical distribution and display genetic features common to all transcriptionally defined GBM subtypes (115). While region-specific differences in the tumor microenvironment likely influence tumor phenotypic and molecular characteristics, the concept of distinct progenitor cell of origin, as a contributing factor to phenotypic and genotypic differences between IDH1 wild-type and mutant GBM, has gained traction (43, 116). The restricted phenotypic and spatial presentation of IDH1 mutant GBM, combined with similarities these tumors share with fetal and adult brain parenchyma, is consistent with their origin from a lineage-committed precursor with limited differentiation potential. These cells are most abundant during a specific stage and location in forebrain development (115), and the exclusive proneural gene expression as well as the oligodendroglial histologic features of IDH1 mutant GBM suggest oligodendrocyte progenitor cells (OPCs) as their cell type of origin (115). Although IDH1 mutation and associated CpG island methylator phenotype (CIMP) together with p53 mutation may occur in quiescent NSCs (cell of mutation), tumors can arise from a lineage-committed progeny (cell of origin), such as OPCs, following proliferative expansion related to forebrain maturation. Mosaic analysis with double markers in a genetically engineered murine GBM model confirmed that mutations originally induced in NSCs can lie dormant and only trigger malignant transformation following differentiation into OPCs, suggesting that mutation may initially occur in either NSCs or OPCs, but only OPCs provide the suitable cellular context needed for transformation (117). Further supporting OPC as a contributor to GBM pathogenesis, OPCs can generate protoplasmic astrocytes, which may allow bipotent OPCs to give rise to brain tumors with astrocytic features. As a result of OPC transformation, low-grade gliomas develop, and through acquisition of additional genomic aberrations, these tumors progress into mutant IDH1 GBM. IDH1 wild-type GBMs, in contrast, show greater variability as concerns anatomical location, histopathological presentation, and genetic profiles, which, together with their molecular resemblance to murine NSCs, point to NSCs as the cell of origin (115). Using Nestin-creERT2, Ascl1-creERT2, and NG2-creERT2 transgenes that drive recombination selectively in NSCs or lineage-restricted progenitor cells, Parada and colleagues (116) demonstrated that distinct GBM-initiating cell populations, in the setting of identical driver mutations, give rise to different GBM subtypes that can be distinguished on histopathological and molecular levels. The tumors showed associations with different types of transcriptome-defined GBM, demonstrated distinct histopathological presentation including varying degrees of necrosis and proliferation, and maintained gene expression profiles specific for the progenitor cell type from which they originate (116). These studies support the idea that the GBM tumors have a “cell of origin memory” that defines tumor phenotypes and genotypes.

When expressed in p53-deficient murine NSCs, mutant IDH1 inhibits progenitor cell growth in vitro, reduces glioma formation in vivo, and increases overall animal subject survival (118). IDH1R132H growth inhibitory effect is due to the diversion of αKG from wild-type IDH1 and reduced carbon flux from glucose or glutamine into lipids. Replenishment of αKG through glutaminolysis compensates for these growth and flux deficiencies (118), suggesting that IDH1 mutant tumors require a specialized metabolic niche characterized by elevated glutamate flux for growth and expansion (Fig. 3). Such a niche is provided by frontal lobe neocortical structures, where most IDH1 mutant tumors occur. Wild-type IDH1 GBM, on the other hand, is unable to sustain high glutamine flux to support αKG production and lipid biogenesis (119). To support anaplerosis in the absence of efficient glutamate catabolism, increased levels of wild-type IDH1 help sustain macromolecular synthesis via enhanced αKG and NADPH production and, in so doing, support wild-type IDH1 GBM growth. Reflecting distinct metabolic wiring in IDH1 mutant versus IDH1 wild-type GBM, NSC and OPC progenitor cells also exist in specific niches within the brain and demonstrate metabolic adaptability to specific and changing microenvironments. OPCs represent the most abundant and widely distributed population of cycling cells in the adult brain. OPCs differentiate into oligodendrocytes, which produce myelin in the CNS, and express glutamate receptors, the activation of which influences numerous cellular processes. Glutamate regulates OPC migration, proliferation, and differentiation; maintains progenitor pools; and ensures sustained neuronal myelination by regulating OPC differentiation into mature oligodendrocytes. In contrast, NSCs are confined to hypoxic subventricular and subgranular zones. NSCs are highly adapted to hypoxia by maintaining low oxidative stress and show increased expression and activity of rate-limiting enzymes implicated in lipogenesis to sustain anaplerosis that supports stem cell expansion (120).

While the studies outlined here point to OPCs as a cell of origin for IDH1 mutant tumors, single-cell RNA sequencing analysis of IDH1 mutant anaplastic astrocytoma and oligodrogliomas points to an NSC/NPC population as the cell of origin. In particular, studies by Suva and co-workers (121, 122) identified three subpopulations of malignant cells in IDH1 mutant gliomas, i.e., nonproliferating differentiated cells with oligodendrocyte- and astrocyte-like glial programs, respectively, and proliferative undifferentiated cells that resemble neural stem/progenitor cells. Future studies using genetically engineered mouse models with progenitor cell–specific IDH1 loss and gain of function will be able to evaluate whether and to what extent IDH1 status, together with the identity of the tumor initiating progenitor cell, influences GBM tumor phenotypes, molecular characteristics, metabolic flux, and therapy susceptibility.

What are the challenges in targeting cancer-associated wild-type IDHs?

Some of the allosteric IDH1R132H inhibitors, including GSK864, have significant IC50 (half-maximal inhibitory concentration) against wild type but only elicit phenotypic changes in cells and derivative tumors when administered at higher micromolar concentrations (69). While the molecular basis for the lack of efficacy is not fully understood, future drug development effort could focus on the elucidation of IDH1 protumor effects that are independent of the enzymatic activity. Furthermore, alternative therapeutic approaches to small-molecule IDH1 inhibitors, including gene regulatory small interfering RNA (siRNA) or microRNA (miRNA), antisense oligonucleotides (ASOs), CRISPR-Cas9 genome editing, and proteolysis targeting chimeras (PROTACs), could be considered to correct IDH1-mediated metabolic rewiring of tumor cells. In addition to targeting wild-type and neomorphic point mutant IDH1 and IDH2 enzymes, the IDH3α:SHMT protein complex emerged as a potential therapeutic target (31). Targeting protein-protein interactions (PPIs) is recognized as a promising therapeutic strategy, and consequently, a broad spectrum of therapeutics, including peptides, monoclonal antibodies, and small molecules, have been explored to disrupt specific PPIs. In particular, interfering peptides (IPs) have been
developed as drug candidates for inhibition of intracellular PPIs, validated for different diseases, including cancer [reviewed in (123)], and advanced into clinical development (123).

Are IDHs risk genes for neurodegeneration?
Functional studies in yeast and drosophila pointed to important pathogenic function of IDHs in neurodegeneration. In particular, IDH loss and ensuing ROS accumulation, mitochondrial damage, and altered lipid metabolism have been implicated in the pathogenesis of PD. A series of epidemiologic studies pointed to low cancer rates in patients diagnosed with neurodegenerative disease, such as PD (124). One hypothesis suggests that the relationship between cancer rates and PD occurrence might be related to the involvement of common genes in both diseases, including parkin, DJ-1, and possibly IDHs. Parkin mutations that alter its activity are linked to PD, while its loss of heterozygosity and copy number have been observed in a spectrum of cancers, such as breast and ovarian cancer (124). Conversely, when overexpressed, DJ-1 and IDHs promote tumor development, while reduced expression is associated with the development of PD. Unraveling the link between IDH expression and function, and the occurrence of neurodegeneration, in particular PD, and cancer may open a therapeutic opportunities to harness IDH metabolic rewiring for the treatment of both diseases. Future genome-wide association studies (GWAS) can determine whether IDHs represent risk loci in neurodegenerative diseases by identifying single-nucleotide variants (SNVs) that are associated with increased disease risk. Functional studies in induced pluripotent stem cells (iPSCs) using CRISPR-Cas9 genome editing to introduce the SNV can then be applied to study SNV impact on IDH expression or activity and can determine upon iPSC differentiation into neurons how specific SNVs affect neuronal function.

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