Published in final edited form as:

Clin Cancer Res. 2016 April 15; 22(8): 1837–1842. doi:10.1158/1078-0432.CCR-13-1333.

Molecular Pathways: Isocitrate Dehydrogenase Mutations in Cancer

Owen Clark[1], **Katharine Yen**[4], and **Ingo K. Mellinghoff**[1,2,3]

[1]Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

[2]Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

[3]Department of Pharmacology, Weill-Cornell Graduate School of Biomedical Sciences, New York, NY 10021, USA

[4]Agios Pharmaceuticals, Cambridge, MA 02139, USA

Abstract

IDH1 and IDH2 are homodimeric enzymes that catalyze the conversion of isocitrate to α-ketoglutarate (α-KG) and concomitantly produce reduced nicotinamide adenine dinucleotide phosphate (NADPH) from $NADP^+$. Mutations in the genes encoding IDH1 and IDH2 have recently been found in a variety of human cancers, most commonly glioma, acute myeloid leukemia (AML), chondrosarcoma, and intrahepatic cholangiocarcinoma. The mutant protein loses its normal enzymatic activity and gains a new ability to produce the 'oncometabolite' R(−)-2-hydroxyglutarate (2HG). 2-HG competitively inhibits α-KG-dependent enzymes which play crucial roles in gene regulation and tissue homeostasis. Expression of mutant IDH impairs cellular differentiation in various cell lineages and promotes tumor development in cooperation with other cancer genes. First-generation inhibitors of mutant IDH have entered clinical trials and have shown encouraging results in patients with IDH2 mutant AML. This article summarizes recent progress in our understanding of the role of mutant IDH in tumorigenesis.

BACKGROUND

The first identification of a cancer-associated *isocitrate dehydrogenase* (*IDH*) mutation was in a patient with colorectal cancer, and emerged from one of the earliest comprehensive analyses of mutations in protein coding genes (1). Two years later, the same group reported a substantially higher frequency of *IDH* mutations (12%) when they applied whole genome sequencing to a small number of glioblastomas (GBMs), the most common malignant brain tumor in adults (2). Interestingly, the majority of *IDH* mutant GBMs (5/6) were from

Corresponding Author: Ingo K. Mellinghoff, 1275 York Avenue, Box 20, New York, NY10021. mellingi@mskcc.org.

Disclosure of Potential Conflicts of Interest

K. Yen has ownership interest (including patents) in Agios Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

patients whose tumors had developed overtime from lower grade (WHO grade II and WHO grade III) tumors. This seminal finding was confirmed in a follow up study with a much larger number of tumors, which reported IDH mutations found in the vast majority (> 70%) of WHO grade II and WHO grade III gliomas (3).

Since these initial studies, many human cancers were examined for the presence of mutations in *IDH1* and *IDH2. IDH* mutations were observed in a number of hematopoietic neoplasms, most commonly acute myeloid leukemia (AML) (~10–15%)(4–6) and angio-immunoblastic T-cell lymphoma (~20%)(7). *IDH* mutations were also found in chondrosarcoma (~50%)(8), intrahepatic cholangiocarcinoma (~15–20%)(9), and - at lower frequency (< 5%) – in other solid tumors (e.g., GBM, colorectal cancer, esophageal cancer, bladder cancer, melanoma, prostate carcinoma, breast adenocarcinoma)(10). Somatic heterozygous mutations in *IDH1* or *IDH2* were also found in the majority of enchondromas and spindle cell hemangiomas in patients with the Ollier disease and Maffuci syndrome, non-hereditary skeletal disorders (11).

More recent DNA resequencing projects have provided additional information regarding the timing of IDH mutations during tumor development. Analyzing over 300 gliomas, Watanabe *et al.* found that in 51 cases with multiple biopsies, neither acquisition of a mutation in *TP53* nor loss of 1p/19q occurred prior to a mutation in *IDH1* (12). Further analysis of matched biopsy pairs, collected from glioma patients at the initial diagnosis and the time of tumor recurrence showed that $IDH1^{R132H}$ was the only mutation that was consistently present in both the initial and recurrent biopsy specimen (13). In leukemia patients, *IDH2* mutations were observed in the absence of *NPM1c* mutations in both mature and progenitor cell populations, suggesting that *IDH2* mutation might be an early and perhaps pre-leukemic event (14, 15).

The vast majority of cancer-associated mutations in *IDH1* and *IDH2* map to an arginine within the catalytic pocket of the enzyme. Mutations in *IDH1* mostly occur at arginine 132, with substitutions including R132H, R132C, R132L, R132S and R132G. Mutations in *IDH2* typically occur at arginine 172 or arginine 140 (which is analogous to R132 in *IDH1*). The clustering of cancer-associated *IDH* mutations in the functional domain of the enzyme suggested that these mutations might endow the mutant protein with a novel and presumably oncogenic enzymatic activity. This question has been explored through untargeted metabolomic profiling of cells engineered to express the mutant enzyme. Compared to parental cells, cells expressing the $IDH1^{R132H}$ mutant enzyme were found to produce the R(−) enantiomer of the metabolite 2-hydroyglutarate (R-2-HG), which accumulates in IDH mutant human gliomas (16) and leukemias (5, 17). Production of R-2-HG involves direct conversion from α-KG and relies on the presence of a wild type allele (18), likely explaining the rareness of loss of heterozygosity.

The identification of an ‘oncometabolite” in IDH mutant tumors strengthened the hypothesis that IDH mutations are oncogenic, and led many investigators to examine the ability of mutant IDH to transform non-malignant cells. Expression of mutant *Idh* in mouse myeloid progenitor 32D cells and primary mouse bone marrow cells impaired hematopoietic differentiation and increased stem/progenitor cell marker expression, suggesting a pro-

leukemogenic effect (19). A more recent study reported that retrovirally mediated expression of mutant *Idh2*^{R140Q} in murine primary hematopoeitic bone marrow stem and progenitor cell populations induced myeloproliferative-like neoplasms, T-cell lymphoma or B-cell lymphoma when transplanted into irradiated mice (20). However, these hematological malignancies occurred at low penetrance and with long latency, suggesting that they did not arise solely due to mutant *Idh2* expression. Expression of mutant *Idh2* in a nontransformed mesenchymal multipotent mouse cell line (C3H, 10T) impaired their differentiation into adipocytic and chondrocytic lineages and resulted in loss of contact inhibition and tumor formation in vivo (21). In immortalized human astrocytes, expression of mutant *IDH*, but not wildtype *IDH* or a catalytically-inactive *IDH* mutant promoted their anchorage-independent growth (22).

Further insights into the role of mutant IDH in tumor initiation have emerged from experiments with genetically engineered mice. Tamoxifen-induced global expression of *Idh2*^{R140Q} or ^{R172K}, driven from the chicken β-actin promoter, resulted in cardiomyopathy, white matter abnormalities throughout the central nervous system, and muscular dystrophy; mice engineered to express mutant *Idh2* in specific tissues reportedly developed carcinomas with very long latencies (23). In another model, mice who expressed a doxycycline-inducible *Idh2*^{R140Q} allele from the Collagen A1 locus did not develop leukemia, even after one year of continuous doxycycline treatment (24). The most common cancer-associated *IDH* mutation, *IDH1*^{R132H}, has also been inserted into the endogenous murine *Idh1* locus and expression of the mutant enzyme subsequently targeted to specific cell populations. Expression of *Idh1*^{R132H} in hematopoietic cells (using the LysM and Vav promoters to express Cre-recombinase) resulted in increased numbers of hematopoietic progenitors, but no overt leukemia (25). Expression of *Idh1*^{R132H} in nestin (*Nes*)-expressing neural stem cells resulted in perinatal lethality due to cerebral hemorrhage (26). Expression of *Idh1*^{R132H} in GFAP-expressing astrocytes resulted in impaired collagen maturation and basement membrane function, but again no tumors. Tamoxifen-inducible expression of mutant *Idh1* in chondrocytes (using the *Col2A1* promoter to express Cre recombinase) resulted in enchondromas, benign cartilage tumors and precursors to malignant chondrosarcomas (27). Doxycycline-induced expression of mutant *Idh2* (*Idh2*^{R140Q} or *Idh2*^{R172K}) increased hepatocyte proliferation in a liver injury-model, but was not sufficient to induce tumors (28).

More recent studies have examined whether IDH mutations might collaborate with other genetic events to induce cancer in mice. Using a mouse transplantation assay, Chaturvedi *et al.* found that mutant *IDH1* was not sufficient to transform hematopoietic cells but accelerated the onset of leukemia in cooperation with *HoxA9* (29). Chen *et al.* applied a mosaic mouse modeling approach in which hematopoietic stem and progenitor cells (HPSC) from *Flt3-ITD* or *Nras*^{G12D} mice were transduced with a retroviral vector expressing mutant *Idh2* and then assessed for tumorigenic potential following transplantation into syngeneic recipient mice. *Idh2* mutants were found to cooperate with *Flt3* or *Nras* alleles to drive leukemia formation (30). Cooperativity between mutant *Idh2*^{R140Q} and other leukemia-relevant pathway alterations (e.g. Flt3-ITD or homeobox proteins HoxA9 and Meis1a) was confirmed in the above-mentioned mouse model (24) and, more recently, another mosaic mouse modeling approach (31). Saha *et al.* showed that *Idh2*^{R172K} cooperated with mutant KRAS (*Kras*^{G12D}) to induce intrahepatic cholangio-carcinomas (28). In aggregate, these

studies demonstrate that mutant IDH cooperates with other oncogenic events to initiate cancer, consistent with the finding that *IDH* mutant human cancers typically harbor alterations in multiple other cancer genes. In glioma, for example, *IDH* mutations are associated with missense mutations in *ATRX*, *TP53*, and *TERT* (diffuse astrocytomas) or co-deletion of chromosome arms 1p and 19q (oligodendrogliomas) (32, 33). In AML patients with a normal cytogenetic profile, *IDH* mutations are associated with mutations in nucleophosmin (*NPM1*), *FLT3-ITD*, and *DNMT3A* (34, 35) (Figure 1).

Recent studies have begun to address the question whether the activity of the mutant IDH enzyme remains important for the growth of IDH mutant cancers once they are fully established. Studies in experimental cancer models suggest that this is indeed the case, with the strongest evidence coming from leukemia models. In TF-1 human erythroleukemia cells, ectopic expression of mutant IDH promotes growth factor independence and this phenotype can be reversed with a small molecule inhibitor of mutant IDH (36, 37). Ex-vivo treatment of freshly isolated IDH mutant leukemic blasts with a mutant selective IDH inhibitor induces a cellular differentiation program (36). Pharmacological inhibition of the mutant IDH enzyme blocks colony formation of human AML cells but not of normal CD34(+) bone marrow cells (29). In a genetically engineered leukemia model, pharmacologic or genetic inhibition of mutant Idh2 triggered the differentiation and death of AML cells (30), and doxycycline-induced silencing of mutant *Idh2* similarly eliminated $Idh2^{R140Q}$/*Hoxa9* or $Idh2^{R140Q}$/*Meis1a*-driven leukemia cells (24).

CLINICAL-TRANSLATIONAL ADVANCES

The above mentioned results in experimental leukemia models are supported by the preliminary findings of an ongoing Phase 1 clinical trial, which showed that the mutant IDH2 inhibitor AG-221 produces clinical responses, including complete and durable responses, in about 40% of patients with AML and MDS (38).

The contribution of the mutant enzyme for the maintenance of IDH mutant solid tumors remains currently unknown and further insights are likely to emerge from an ongoing single-arm dose escalation study (ClinicalTrials.gov NCT02073994) with the mutant-selective IDH1 inhibitor AG-120 (39). Data from experimental models suggest that *IDH1*-mutant solid tumors remain, at least in part, dependent on the activity of the mutant enzyme, In HT1080 human fibrosarcoma cells, RNAi-mediated suppression of endogenous mutant *IDH1* significantly inhibited anchorage-independent growth (40). Knocking out the endogenous mutant *IDH1* gene using TALEN technology similarly impaired anchorage-independent growth and *in-vivo* growth of *IDH*-mutant human sarcoma cells (41). In *IDH1* mutant glioma cells, pharmacological blockade of the mutant enzyme retarded their growth in soft-agar and in mice (42).

Targeted therapy of *IDH* mutant cancers is likely to grow beyond inhibition of the mutant enzyme itself and may include strategies directed against the epigenetic and metabolic changes that are associated with IDH mutations. Current data suggests that the R-2-HG ‘oncometabolite’ is responsible for many, if not all, biological effects of cancer-associated IDH mutations. Cell-permeable esters of R-2-HG phenocopy the effects of mutant *IDH* in a

range of experimental models (43) and ectopic expression of the dehydrogenase (44) that converts R-2-HG into α-KG and thus counteracts the activity of the mutant IDH enzyme is sufficient to reverse the cellular effects of cancer-associated *IDH* mutants (45). R-2-HG competitively inhibits a large family of α-KG-dependent enzymes, a protein family with over 60 members. These include the ten-eleven translocation (TET) family of 5-methyl cytosine hydroxylases, the jumonji domain containing (JmjC) family of histone lysine demethylases (46, 47), enzymes involved in nucleic acid metabolism (AlkB, FTO), and many enzymes with still unknown functions (48) (Figure 2).

It is currently unknown which α-KG-dependent enzymes function as context-dependent tumor suppressors and are inhibited at the relevant R-2-HG concentrations in human tumors. Inhibition of TET2 is likely to mediate the effects of mutant *IDH* in AML, given the well documented role of TET2 in hematopoietic differentiation, the almost completely mutual exclusivity of *TET2* and *IDH* mutations in AML (Figure 1), the decreased 5-hydroxymethylcytosine levels in *IDH* mutant AMLs, and the identification of TET2 as candidate effector of mutant IDH in a short hairpin RNA library screen of α-KG-dependent dioxygenases (19, 37, 49–51). Inhibition of the histone demethylases JMJD2A and JMJD2C likely contributes to the effects of mutant *IDH* on cellular differentiation in many cell lineages. These enzymes are particularly sensitive to inhibition by R-2-HG (46, 47), and knockdown of *Jmjd2C* (also known as *Kdm4c*) was sufficient to phenocopy the effects of mutant *Idh* on adipocyte differentiation in 3T3-L1 cells (43). In fact, it seems plausible than many hallmarks of mutant *IDH*-associated human malignancies, such as restricted cellular differentiation, DNA hypermethylation result from coordinate effects of R-2-HG on DNA and histone methylation (43, 52). These findings raise the intriguing question of whether *IDH* mutant cancers cells might show an increased sensitivity to the DNA methyltransferase inhibitors (DNMTIs) decitabine or 5-azacytidine (53–55) or drugs targeting histone modifications.

It is less clear whether epigenetic alterations are also responsible for the oncogenic effects of mutant *IDH* in solid tumors. We observed that growth inhibition of *IDH1* mutant glioma xenografts by a mutant selective IDH1 inhibitor was not clearly linked to epigenetic changes in tumor tissue (42). Similarly, ectopic expression of the R-2-HG-dehydrogenase in HT1080 sarcoma cells effectively inhibited their growth *in vivo* without clear changes in histone methylation marks or TET2-induced 5-hmC production (41). It is plausible that the oncogenic effects of mutant *IDH* in solid tumors are linked to metabolic alterations associated with *IDH* mutations including reductions in α-KG levels, changes in the $NADP^+$/NADPH ratio and mitochondrial bioenergetics, and an impaired ability of the IDH enzyme to catalyze the reverse carboxylation of α-KG to form isocitrate. These metabolic changes can have a profound effect on the ability of cells to proliferate, differentiate, and escape cell death, and may introduce therapeutic vulnerabilities (56–64). Recent studies reported an enhanced susceptibility of *IDH1* mutant cancers to inhibitors of BCL-2 (63), due to the effect of mutant IDH on mitochondrial bioenergetics, or to depletion of the coenzyme NAD + (65).

The success of therapeutic strategies to curb the growth of *IDH* mutant cancers will hinge on a deeper understanding of the molecular mechanisms of *IDH*-associated tumorigenesis and

R-2-HG effector pathways in each cancer type. In addition to the epigenetic and metabolic effects of mutant *IDH* outlined above, the (R)-enantiomer of 2-hydroxyglutarate has been reported to stimulate the activity of the EGLN prolyl 4-hydroxylases, leading to diminished levels of hypoxia-inducible transcription factor (HIF) and enhanced soft agar growth of human astrocytes (66). Consistent with this result, the expression of HIF1α-responsive genes, including many essential for glycolysis appears to be lower in *IDH* mutant gliomas (67), perhaps explaining the often poor retention of the radiotracer 2-[(18)F] Fluoro-2-deoxy-D-glucose (FDG) by these tumors. The group of α-KG-dependent dioxygenases also includes enzymes that hydroxylate proline and lysine residues in collagen and promote its stability. It is intriguing to speculate that the observed brain hemorrhages in *Nes-Cre Idh1*^{R132H} mice might have resulted from impaired collagen maturation and basement membrane architecture (68).

A remarkable body of knowledge has been generated in the few years since the first description of cancer associated *IDH* mutants and the future seems bright for the development of rationale therapeutic approaches of *IDH* mutant cancers. The ability to monitor the activity of the mutant IDH enzyme through quantification of 2-HG levels in blood (69) or Magnetic Resonance Spectroscopy in tumors (70–72) will be instrumental to develop such strategies.

Acknowledgments

GRANT SUPPORT

Research reported in this publication was supported by the NIH under award numbers R01NS080944 (to I.K. Mellinghoff), T32CA160001 (to O. Clark), and P30CA008748 (to O. Clark and I.K. Mellinghoff, through their institution). I.K. Mellinghoff was supported by the Ben and Catherine Ivy Foundation and the Defeat GBM Research Collaborative of the National Brain Tumor Society.

The authors wish to thank Dr. Sara Kubek for her critical input to graphic illustration, as well Drs. Donna Nichol and Paolo Codega for help and advice with the manuscript.

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Figure 1. Co-occuring genetic lesions in *IDH*-mutant glioma and AML

Oncoprint figure (generated using Memorial Sloan Kettering cBio Portal)(10) showing frequency of commonly co-occuring genetic lesions in glioma (top) and AML (bottom). Note that *TET2* is shown to illustrate near complete mutual exclusivity with mutations in *IDH1/2*. Results are based upon data generated by the TCGA Research Network: Glioma data set (32); AML data set (73). For simplicity changes in copy number were not included. *TET2 - Tet Methylcytosine Dioxygenase 2; DNMT3A - DNA (Cytosine-5-)-Methyltransferase 3 Alpha; FLT3 - Fms-Related Tyrosine Kinase 3; NPM1 - Nucleophosmin (Nucleolar Phosphoprotein B23, Numatrin); TP53 - Tumor Protein P53; ATRX - Alpha Thalassemia/Mental Retardation Syndrome X-Linked*.

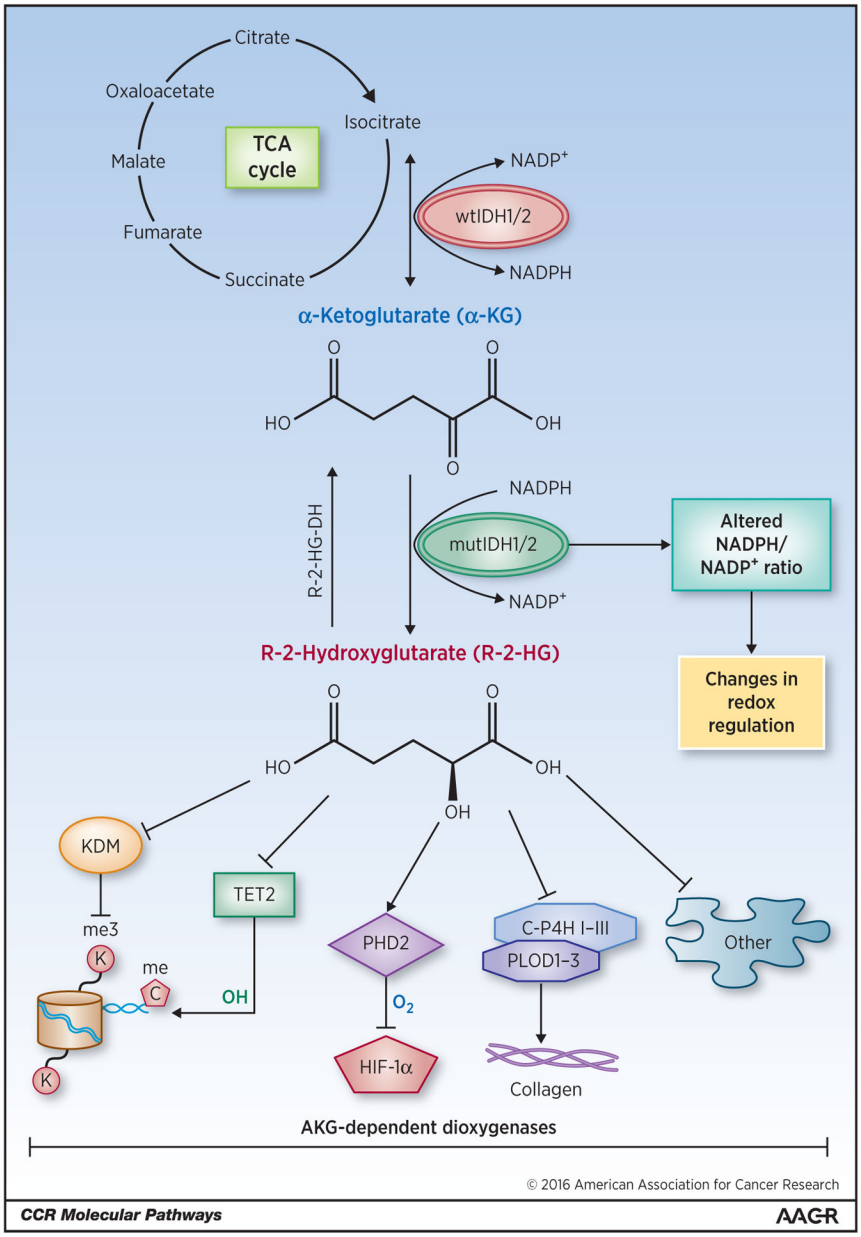

Figure 2. Molecular mechanisms of *IDH*-associated tumorigenesis

Wild-type IDH1/2 (wtIDH1/2) converts isocitrate (generated through the citric acid (TCA) cycle) into α-KG, producing NADPH in the process. Mutant IDH1/2 (mutIDH1/2) converts α-KG to the oncometabolite R-2-HG, consuming NADPH. R-2-HG inhibits members of the protein family of α-KG-dependent dioxygenases. Epigenetic modifications result from inhibition of histone lysine demethylases (KDMs) and the 5-methyl cytosine hydroxylase TET2. Inhibition of prolyl-hydroxylation impairs collagen maturation. R-2-HG has been reported to activate the enzyme prolyl-hydroxylase 2 (PHD2) that inhibits hypoxia-inducible factor 1-alpha (HIF-1α) (22), although other studies suggest that it may be inhibited (46,

47). Mutant IDH may also change the cellular redox environment by altering the ratio of NADPH to $NADP^+$. IDH3 has been omitted from this figure for simplicity.