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Review

Oncogenic Activities of IDH1/2 Mutations: From Epigenetics to Cellular Signaling

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Gliomas and leukemias remain highly refractory to treatment, thus highlighting the need for new and improved therapeutic strategies. Mutations in genes encoding enzymes involved in the tricarboxylic acid (TCA) cycle, such as the isocitrate dehydrogenases 1 and 2 (IDH1/2), are frequently encountered in astrocytomas and secondary glioblastomas, as well as in acute myeloid leukemias; however, the precise molecular mechanisms by which these mutations promote tumorigenesis remain to be fully characterized. Gain-of-function mutations in IDH1/2 have been shown to stimulate production of the oncogenic metabolite *R*-2-hydroxyglutarate (*R*-2HG), which inhibits α-ketoglutarate (αKG)-dependent enzymes. We review recent advances on the elucidation of oncogenic functions of IDH1/2 mutations, and of the associated oncometabolite *R*-2HG, which link altered metabolism of cancer cells to epigenetics, RNA methylation, cellular signaling, hypoxic response, and DNA repair.

Trends

Isocitrate dehydrogenase 1 and 2 (*IDH1/2*) mutations are frequent in brain cancer, acute myeloid leukemia, and other cancers, and are associated with a better prognosis in both astrocytomas and glioblastomas.

Mutations in IDH1/2 occur at specific amino acid residues and cause gain-of-function leading to the production and accumulation of the oncometabolite *R*-2HG.

R-2HG regulates the activity of numerous αKG-dependent enzymes involved in epigenetic regulation, chromatin modifications, RNA methylation, mTOR signaling, response to hypoxia, and collagen maturation.

Pharmacological inhibitors specific to the mutated forms of IDH1/2 efficiently diminish *R*-2HG levels and allow differentiation of cancer cells, and are currently under clinical investigation.

IDH1/2 Gain-of-Function Mutations in Cancer

It has become evident that interference with normal metabolic function is a hallmark of cancer [1]. As one prominent example, enzymes involved in the TCA cycle including isocitrate dehydrogenases 1 (IDH1) and 2 (IDH2), are frequently mutated in some tumor types. Heterozygous *IDH1/2* mutations are recovered in 70–80% of WHO grade 2 and 3 astrocytomas, oligodendrogliomas, and secondary glioblastomas [2], as well as in ~20% of acute myeloid leukemias (AML) [3–5]. Moreover, *IDH1/2* mutations have been identified in chondrosarcoma [6–8], cholangiocarcinoma [9], osteosarcoma [10], and prostate cancer [11,12], as well as in Ollier disease and Maffucci syndrome [7,13] which are associated with benign cartilaginous or vascular lesions, respectively. Although glioblastoma patients carrying *IDH1/2* alterations display improved prognosis, their median overall survival is only approximately 31 months for gliomas [14] and 44 months for glioblastoma multiforme (GBM) [15]. Interestingly, *IDH1* mutations constitute an early event in gliomagenesis [16,17], and have been observed in pre-leukemic cells [18,19], and therefore may play a crucial role in initiating the disease. In addition, *IDH1/2* mutations *in vivo* contribute to the development of gliomas, leukemias, cholangiocarcinomas, and sarcomas by increasing the number of stem cells and impairing differentiation [20–25].

Wild-type IDH1/2 catalyzes the formation of αKG from isocitrate (Figure 1). Cancer-associated IDH1/2 mutations occur most commonly at substrate-binding amino acid residues (Arg132 for

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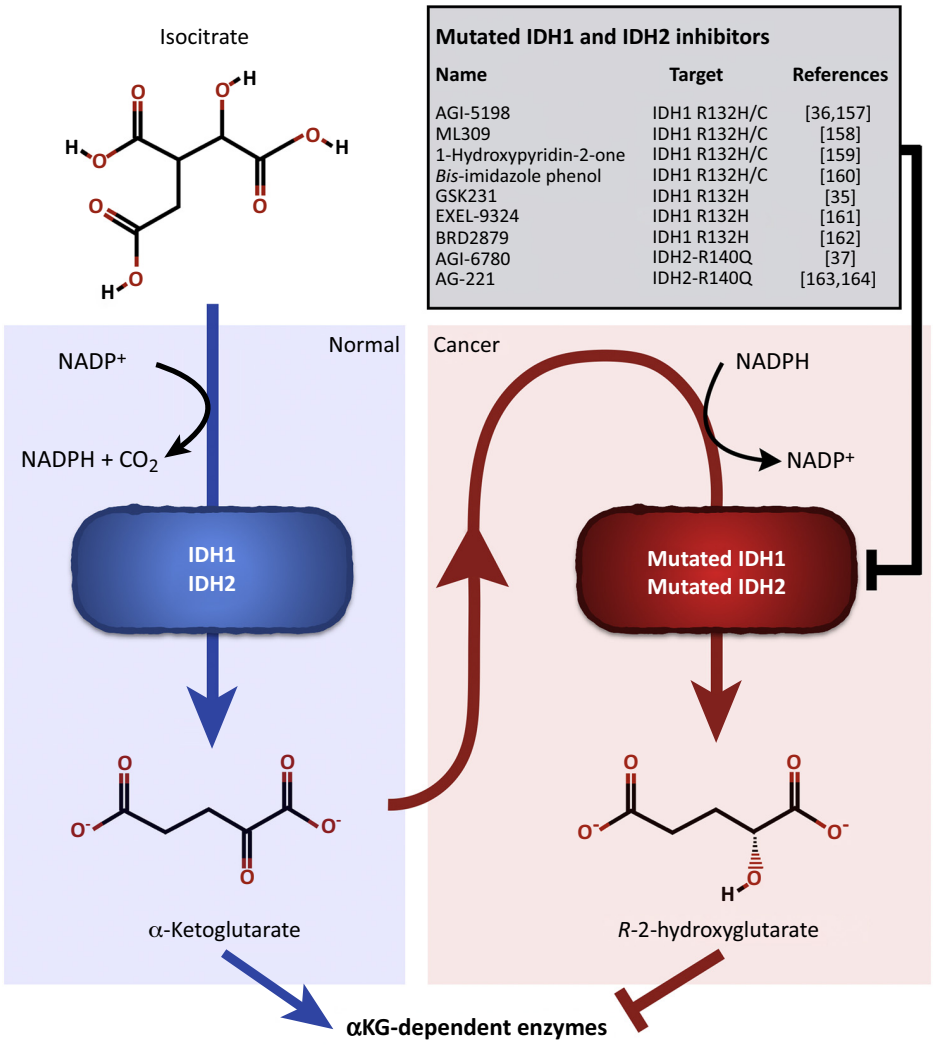

Figure 1. Mutant IDH1 and IDH2 Catalyze the Production of *R*-2-Hydroxyglutarate (*R*-2HG) from α-Ketoglutarate (αKG). Cancer-associated mutations identified in IDH1/2 enzymes confer a specific gain-of-function promoting the production of *R*-2HG, an oncometabolite that contributes to transformation. This new metabolic function is achieved by using αKG, the product of wild-type IDH1/2, potentially explaining the heterozygosity of *IDH1/2* mutations. IDH1/2 mutations might decrease the availability of αKG for α-KG dehydrogenase, the enzyme of the tricarboxylic acid (TCA) cycle that catalyzes the production of succinyl-CoA from αKG, resulting in a gradual decrease of TCA cycle metabolites [156]. Accumulation of *R*-2HG inhibits numerous αKG-dependent dioxygenases which are involved in various cellular and biological pathways. Conversely to wild-type IDH1/2, neomorphic IDH1/2 mutants consume NADPH during the conversion of αKG into *R*-2HG, thus allowing sustained availability of $NADP^+$ for the production of αKG from isocitrate by the wild-type IDH1/2 allele. Several inhibitors of the mutated forms of IDH1/2 have been identified [35–37,157–164] and some are in clinical trials [137].

IDH1; Arg140 and 172 for IDH2), resulting in abrogation of αKG production. Moreover, these mutations engender a gain-of-function leading to the formation and excessive accumulation of *R*-2-hydroxyglutarate (*R*-2HG) (Box 1) [5,26]. *R*-2HG was therefore proposed to act as an 'oncometabolite' [27]. *R*-2HG accumulates readily in brain tumors harboring *IDH1/2* mutations and can be detected by magnetic resonance spectroscopy in glioma patients [28–30]. This has provided a useful non-invasive tool for monitoring progression of the disease post-treatment

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Box 1. The Metabolism of Enantiomers of 2-Hydroxyglutarate

Owing to the presence of a chiral carbon, 2-hydroxyglutarate (2HG) exists as two enantiomers: *R*-2HG (or *D*-2HG) and *S*-2HG (or *L*-2HG). In addition to the mutated forms of IDH1/2 [26], wild-type IDH2 [141] and *D*-3-phosphoglycerate dehydrogenase (PHGDH) [142] also catalyze the reduction of αKG into *R*-2HG. Accordingly, *PHGDH* is frequently amplified in breast cancer [143], and a subset of breast malignancies have been shown to accumulate 2HG [144]. Conversely, *S*-2HG is generated via promiscuous substrate utilization by lactate dehydrogenase A (LDHA) and malate dehydrogenase (MDH), and accumulates during hypoxia [145,146], in acidic conditions [147,148], as well as during *Drosophila* development [149]. In addition, *S*-2HG is produced in activated $CD8^+$ T lymphocytes and stimulates their proliferation and survival [150]. Similarly to *R*-2HG, *S*-2HG can inhibit numerous αKG-dependent enzymes [38,39]. Furthermore, kidney tumors display high levels of *S*-2HG, suggesting a potential contribution of this oncometabolite to renal carcinogenesis [151]. Both *R*-2HG and *S*-2HG can be converted back to αKG by D-2-hydroxyglutarate dehydrogenase (D2HGDH) and L-2-hydroxyglutarate dehydrogenase (L2HGDH), respectively. Germline loss of functional *D2HGDH* or *L2HGDH* is associated with accumulation of 2HG resulting in congenital aciduria [152,153] and, in the case of *S*-2HG-linked aciduria, increased risk of brain cancer. *L2hgdh* knockout mice display accumulation of *S*-2HG in the brain and features of neurodegeneration [154]. Germline gain-of-function mutations of *IDH2* are also associated with *R*-2HG aciduria [155], suggesting that endogenous D2HGDH is not sufficient to cope with *R*-2HG levels in this specific context.

[31]. *R*-2HG is also significantly elevated (~50-fold) in the sera of AML patients carrying *IDH1/2* mutations, allowing predictive identification of *IDH1/2* mutation and clinical outcome in AML [32,33]. *R*-2HG is sufficient to promote transformation of hematopoietic cells, and this effect is reversible upon removal of the oncometabolite [34]. Pharmacological inhibitors that efficiently diminish *R*-2HG production in *IDH1/2* mutated cells have been developed [35–37] and are currently under clinical investigation. Nonetheless, the diverse molecular mechanisms by which *R*-2HG promotes tumorigenesis remain poorly understood. The structural similarity between *R*-2HG and αKG allows the former to act as an inhibitor of αKG-dependent enzymes [38,39], of which ~60 have been described to date in humans [40]. Members of the αKG-dependent dioxygenases include several subfamilies such as the Jumonji family of lysine demethylases, prolyl hydroxylases, and the ten-eleven translocation (TET) family of 5-methylcytosine hydroxylases. Dysregulation of αKG-dependent dioxygenases is linked to both cancer initiation and progression. Of note, the different αKG-dependent dioxygenases exhibit a wide range of sensitivities to *R*-2HG [40]. Because intratumoral concentrations of *R*-2HG attain millimolar levels in *IDH1/2* mutated tumors and xenografts [26,36], even the less-sensitive αKG-dependent dioxygenases might be subject to *R*-2HG inhibition in such settings. Because αKG-dependent enzymes are involved in modulating chromatin structure, DNA/RNA methylation, cellular signaling, response to hypoxia response, and collagen maturation, cancer cells carrying *IDH1/2* mutations face potential dysregulation of these crucial processes. We provide here an overview of the different oncogenic modes of action of *IDH1/2* mutations and *R*-2HG.

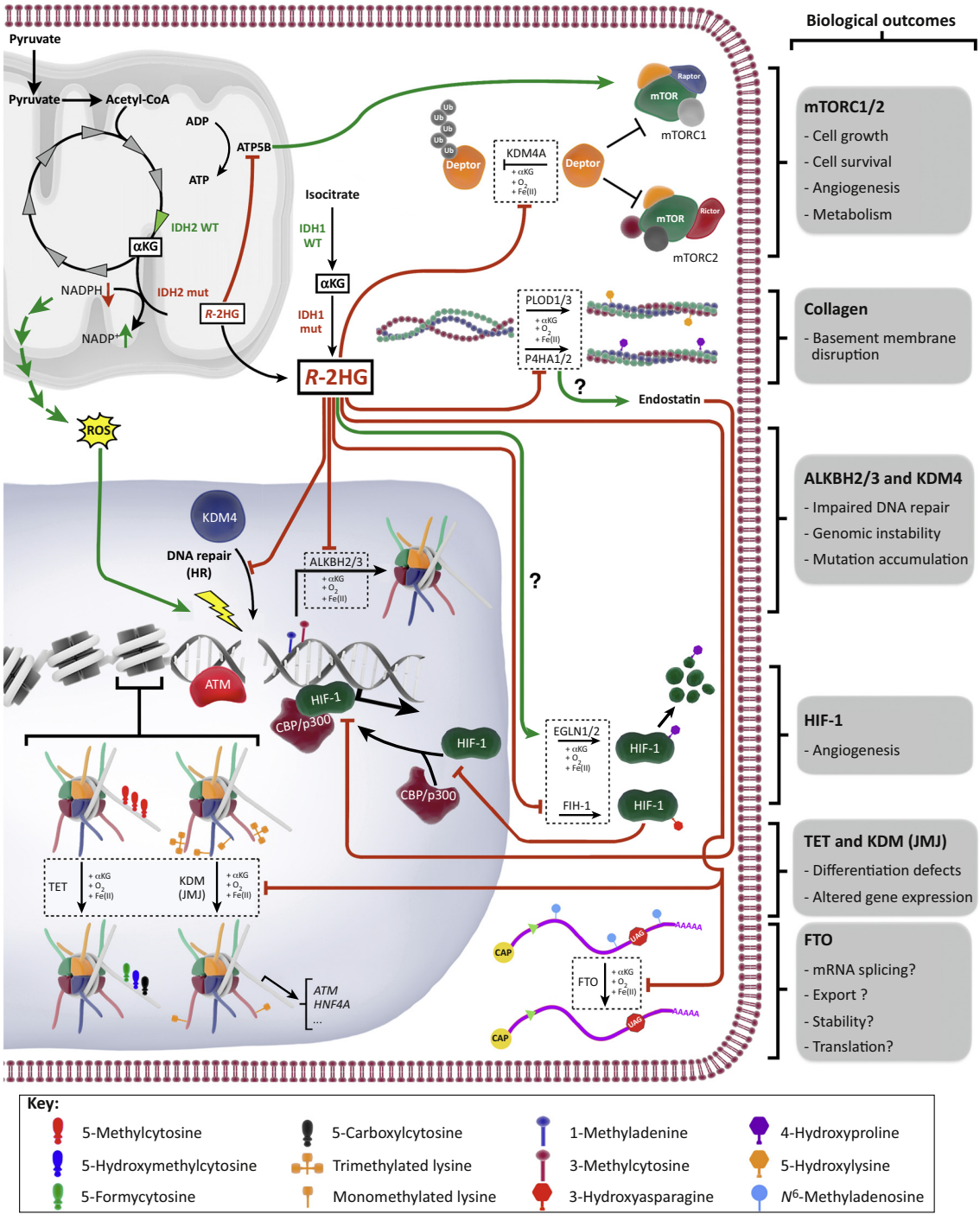
R-2HG and Altered Epigenetics

Epigenetic modifications, such as methylation of DNA and histones, regulate gene expression. DNA hypermethylation of promoters leads to transcriptional silencing of crucial tumor-suppressor genes during cancer development [41].

The TET family (TET1, TET2, and TET3) of αKG-dependent dioxygenases catalyze the hydroxylation of 5-methylcytosine into 5-hydroxymethylcytosine [42,43], 5-formylcytosine, and 5-carboxycytosine [44]. TET-mediated modifications are required for subsequent demethylation of 5-methylcytosine [45]. *TET2* mutations, occurring in ~15% of myeloid cancers [46], are generally mutually exclusive with *IDH* mutations [47]. *R*-2HG produced by gain-of-function IDH1/2 mutations inhibit TET2 catalytic activity [39] (Figure 2, Key Figure; Table 1). Accordingly, a glioma CpG island methylator phenotype has been associated with *IDH1* mutations in tumor samples [48]. Further evidence demonstrated that $IDH1^{R132H}$ mutation triggers the hypermethylator phenotype in gliomas [49,50]. In hematopoietic cells, mutated *IDH1/2* also leads to a hypermethylation phenotype, resulting in differentiation defects [47]. For example,

Key Figure

Cellular Processes Modulated by the *R*-2-Hydroxyglutarate (*R*-2HG)-Mediated Regulation of α-Ketoglutarate (αKG)-Dependent Dioxygenases (Epigenetic Modifications)



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transcriptional silencing of tumor-suppressor genes by DNA hypermethylation is likely to increase progenitor cell numbers with extended proliferative potential. Treatment of IDH1/2 mutated cells with either small-molecule inhibitors of mutated IDH or DNA methyltransferases (DNMTs) reversed the DNA hypermethylation phenotype, restored expression of differentiation genes, and promoted cell differentiation [51–53].

Chromatin can form complex structures and loops the generate topologically associated domains with regulatory function. The CTCF insulator protein binds to DNA to form boundaries between such domains, regulating the function of enhancers and modulating gene expression [54]. Because CTCF binding to insulator domains is compromised by DNA methylation [55,56], DNA hypermethylation occurring in *IDH1/2* mutated cells alleviates the binding of CTCF and allows the activation of enhancers stimulating the expression of oncogenes such as *PDGFRA*, encoding a receptor tyrosine kinase that is frequently activated in gliomas [57].

Overall, mutated IDH1/2-mediated inhibition of TET enzymes causes alteration of gene expression through aberrant DNA methylation. The complete gene network dysregulated by DNA hypermethylation, as well as the function of these genes in cancer initiation and progression in *IDH1/2* mutated cancers, remain to be fully understood.

In addition to DNA methylation, lysine methylation of histone tails also influences chromatin structure and regulates gene expression. Jumonji C-domain lysine demethylases are αKG-dependent dioxygenases sensitive to *R*-2HG [38,39]. Ectopic expression of IDH1/2 mutants leads to dramatic accumulation of lysine-methylated histones (trimethylated histone H3 on lysine 9, H3K9me3; and on lysine 27, H3K27me3, among others) [23]. H3K9me3 and H3K27me3 are also elevated in *IDH1* mutated oligodendroglioma compared to wild-type *IDH1/2* [23]. Mutant IDH2 or cell permeable *R*-2HG impairs adipocyte differentiation by increasing H3K9me3 and H3K27me3 at promoters of key genes promoting differentiation, thus decreasing their transcription. Depletion of the *R*-2HG-sensitive H3K9me3 demethylase KDM4C similarly blocked adipocyte differentiation, suggesting a role for inhibition of this demethylase by *IDH1/2* mutations in preventing differentiation [23]. Further supporting the capacity of *IDH* mutations to block differentiation, $IDH1^{R132C}$ hinders transcription of *HNF4A*, a

Figure 2. Multiple αKG-dependent dioxygenases family are dependent on αKG for their enzymatic activities to modify gene expression. When associated with αKG, ten-eleven translocation (TET) family catalyze the conversion of the modified DNA base 5-methylcytosine to 5-hydroxymethylcytosine, which can then be further oxidized to form derivatives 5-formylcytosine and 5-carboxylcytosine. While this activity requires αKG, TET methylcytosine hydroxylation is strongly inhibited by *R*-2HG production following *IDH1/2* mutation. Similarly, the activity of Jumonji lysine demethylases, which normally demethylate specific regulatory lysines on histones H3 and H4, is abrogated upon *R*-2HG production, and the ensuing major changes in gene expression impair cell differentiation. (RNA methylation) FTO (fat mass and obesity-associated protein) catalyzes the demethylation of N^6-methyladenosine (m^6A), an abundant modification present in mRNAs. m^6A regulates several aspects of RNA metabolism such as RNA splicing, transport, stability, and translation. FTO may be inhibited by *R*-2HG, but the consequences of reduced FTO activity in mutant IDH tumors are largely unknown. (DNA damage response and repair) ALKBH2 and ALKBH3 (α-KG-dependent dioxygenase homologs 2 and 3) are involved in reparation of alkylated DNA lesions containing 1-methyladenine and 3-methylcytosine by oxidative demethylation. *R*-2HG production inhibits ALKBH2/3 activity, thus interfering with normal DNA repair processes. Inhibition of KDM4A and KDM4B by *R*-2HG leads to DNA repair defects by impairing homologous recombination (HR). In addition, IDH mutations cause accumulation of the repressive H3K9me3 histone mark at the promoter of gene encoding the DNA damage response kinase ATM, thus decreasing the levels of ATM and altering DNA repair. (mTOR Signaling) KDM4A and ATP5B modulate mTOR activation in the presence of αKG or *R*-2HG. KDM4A stabilizes DEPTOR, the endogenous negative regulator of mTOR, by inhibiting its polyubiquitylation, thus decreasing both mTORC1 and mTORC2 activation. In presence of *R*-2HG, DEPTOR is rapidly degraded, resulting in a massive increase in mTORC1/2 activation in a PI3K/PTEN-independent fashion, thus favoring the proliferation and survival of cancer cells harboring IDH1/2 mutations. In addition, *R*-2HG inhibits ATP synthase activity, leading to reduced ATP content and thus causing mTOR inhibition. *R*-2HG production by *IDH1/2* mutations engages a complex network that likely regulates mTOR activity through regulatory feedback loops. (HIF-1 Signaling) Hypoxia-inducible factor 1α (HIF-1α) is a major regulator of cell proliferation, cell survival, and a promoter of angiogenesis. HIF-1α is a highly unstable protein regulated by O_2 availability. EGLN1/2 and FIH are potent regulators of HIF-1α stability and activity, respectively. While FIH is inhibited by *R*-2HG, EGLN1/2 may be activated by the oncometabolite, resulting in a complex modulation of HIF-1α functions in tumors carrying *IDH1/2* mutations. (Collagen maturation) Both prolyl 4-hydroxylases (P4HA1/3) and procollagen-lysine 2-oxoglutarate 5-dioxygenases (PLOD1/3) are enzymes that stabilize the collagen triple helix through hydroxylation of lysine and proline within each collagen chain. Because these enzymes are αKG-dependent, reduced availability of αKG and accumulation of *R*-2HG in *IDH1/2* mutated tumors affect collagen maturation.

Table 1. Enzymes Regulated by Mutated IDH1/2 and the Associated Oncometabolite *R*-2HG

Enzyme	Enzymatic function	Type of regulation by *R*-2HG	Consequence of *R*-2HG-mediated regulation
TET1	Methylcytosine dioxygenase	Inhibition [39]	Inhibition of TET1 and TET2 by IDH1/2 mutations caused downregulation of 5-hydroxymethylcytosine (5hmC) [39]
TET2	Methylcytosine dioxygenase	Inhibition [39]	Inhibition of TET1 and TET2 by IDH1/2 mutations caused downregulation of 5hmC [39]; impaired hematopoietic differentiation [47]
KDM2A	H3K36me1/2 demethylase	Inhibition (IC_{50} = 106 μM [39])	Not determined
KDM4A	H3K9me2/3, H1.4K26me2/3, and H3K36me2/3 demethylase	Inhibition (IC_{50} from 2.1 μM [97] to 24 μM [38])	Global increase of H3K9me3 [38]; increased mTOR activity through destabilization of DEPTOR [97]; DNA repair defects [82]
KDM4C	H3K9me2/3, H1.4K26me2/3, and H3K36me2/3 demethylase	Inhibition [23]	Global increase of H3K9me3 [38]; differentiation defects [23]
KDM4D	H3K9me2/3, H1.4K26me2/3, and H3K36me2/3 demethylase	Inhibition (IC_{50} = 79 μM [38])	Global increase of H3K9me3 [38]
KDM5B	H3K4me2/3 demethylase	Weak inhibition (IC_{50} = 10.9 mM [38])	Not determined
HIF1AN/FIH	HIF asparaginyl hydroxylase	Inhibition (IC_{50} from ~1 mM [123] to 1.5 mM [38])	HIF-1α accumulation in *R*-2HG-treated cells [39] as well as in $IDH1^{R132H}$-expressing cells and gliomas [116]
EGLN1/2	HIF prolyl 4-hydroxylase	Activation [123]	Activation of EGLN1/2 in IDH^{R132H} mutated cells diminished HIF activity [123]
Collagen prolyl hydroxylases (P4HA1/2) and lysine hydroxylases (PLOD1/3)	Collagen prolyl hydroxylases; Collagen lysine hydroxylases	Inhibition (IC_{50} ~2 mM; C-P4H-I [123])	Defective collagen maturation, impairment of basal membrane structure and function, activation of the ER stress response [92]
ALKBH2	Repair of methylated DNA bases, such as 1-methyladenine and 3-methylcytosine	Inhibition (IC_{50} from 424 μM [38] to ~500 μM [83])	Accumulation of DNA damage, decreased DNA repair kinetics, and increased sensitivity to alkylating agents of IDH mutated cells [83]
ALKBH3	Repair of methylated DNA bases such as 1-methyladenine and 3-methylcytosine	Inhibition (IC_{50} ~500 μM [83])	Accumulation of DNA damage, decreased DNA repair kinetics, and increased sensitivity to alkylating agents of IDH mutated cells [83]
FTO	N^6-methyladenosine and N^6,2′-*O*-dimethyladenosine RNA demethylase	Inhibition [70]	Accumulation of m^6A RNAs [70]
ATP5B	ATP synthase β subunit	Inhibition [104]	Increased ratio of ADP to ATP, inhibition of mTOR signaling [104]

master regulator of hepatocyte differentiation [24]. In hepatocytes, $IDH1^{R132C}$ decreases levels of H3K4me3, a histone mark associated with active transcription, at the promoter of *HNF4A*. IDH-mediated impairment of differentiation cooperated with *RAS* mutations to drive intrahepatic cholangiocarcinoma [24].

Altogether, *R*-2HG alters gene expression by modifying the pattern of histone lysine methylation, suggesting that small-molecule inhibitors of histone methyltransferases could exhibit therapeutic potential by restoring physiological levels of histone methylation. While it is clear that IDH1/2 mutations and *R*-2HG inhibit Jumonji C-domain lysine demethylases *in vitro*, the relative sensitivity of these enzymes to *R*-2HG *in vivo* remains unknown. Furthermore, the loci mostly

affected by *R*-2HG-mediated inhibition of lysine demethylases, as well as the nature of the dysregulated genes and their contributions to IDH1/2-mediated transformation, requires additional investigation.

R-2HG-Mediated Inhibition of RNA Demethylases

N^6-adenosine methylation (m^6A) is an abundant modification modulating several aspects of post-transcriptional control including RNA splicing, export, stability, and translation [58–64]. m^6A is catalyzed by a multisubunit complex comprising the RNA methyltransferase METTL3, METTL14, the Wilms tumor-associated protein (WTAP), and RBM15 [61,65–68]. Conversely, m^6A is reversed by two αKG- and iron-dependent dioxygenases, FTO (fat mass and obesity-associated protein) and ALKBH5 [63,69].

A recent study reported that cells overexpressing mutant IDH as well as primary IDH mutant AML samples displayed increased m^6A levels, likely a consequence of FTO inhibition by *R*-2HG [70]. Interestingly, higher expression of the methyltransferase complex proteins was observed in myeloid leukemia [71,72] and in glioblastoma [73]. It remains unknown whether the upregulation of the components of the methyltransferase machinery in these tumor types results in increased m^6A marks as seen in the context of IDH mutation. By contrast, FTO is highly expressed in AML carrying *MLL* rearrangements, *PML–RAR*, *FLT3-ITD*, or *NPM1* mutations, and seems to play oncogenic roles in these settings [74]. It remains to be determined whether FTO behaves as a context-dependent oncogene or FTO inhibition by *R*-2HG contributes to a potentially less aggressive disease in IDH mutated cancers. Hence, to fully understand the contribution of FTO inhibition in IDH mutant cancers, it will be important to identify the targets of altered methylation events and define the functional consequences of m^6A dysregulation on the fate of those transcripts. Of note, it has been recently reported that FTO impacts on mRNA stability by targeting methylated adenosines following the 5′ mRNA cap (N^6,2′-*O*-dimethyladenosine) but not m^6A in the body of mRNA as was previously thought [75]. In the light of these findings, it appears that further investigation will be necessary to establish the molecular underpinnings of the role of FTO in neoplasia.

DNA Damage Response and DNA Repair Defects Caused by *IDH1/2* Mutations

DNA damage can be induced exogenously by a plethora of environmental mutagens, or endogenously during normal metabolism by reactive oxygen species (ROS), alkylating agents, or interstrand crosslinkers. Failure to recognize or repair damaged DNA causes genomic instability leading to genetic or epigenetic alterations in crucial oncogenes and tumor-suppressor genes (e.g., *TP53*, *RB*, *RAS*/*RAF*, *PTEN*/*AKT*, and *TERT*) thus driving multistage carcinogenesis [1]. The PI3K-like kinase ataxia-telangiectasia mutated (ATM) constitutes a crucial activator of the DNA damage response and triggers a complex signaling network which culminates in the recruitment of repair factors to DNA double-strand breaks [76]. ATM also plays a crucial role in many other cellular processes including maintenance of the self-renewal capacity of hematopoietic stem cells [77]. Knock-in mice expressing mutated IDH1 in the myeloid lineage exhibit a global decrease in the ATM phosphorylation cascade in various hematopoietic stem and progenitor cells, including autophosphorylation of ATM and phosphorylation of the ATM downstream effectors CHK2 and histone variant H2AX (γH2AX) [78]. Expression of mutated IDH1 in the hematopoietic system caused a modest but significant downregulation of *Atm* mRNA, associated with a dramatic decrease of ATM protein [78]. This was not recapitulated in *Tet2* knockout animals, suggesting a TET2-independent mode of regulation of ATM by *R*-2HG. A more condensed chromatin structure surrounding the *Atm* promoter, as well as increased H3K9 methylation, was observed in *Idh1* mutated cells. Although it remains to be confirmed, inhibition of the KDM4 family of demethylases might

be responsible for the accumulation of H3K9 methylation because these enzymes are highly sensitive to *R*-2HG [38]. Pharmacological inhibition of the H3K9 methyltransferase G9a restored ATM protein and mRNA levels in *Idh1* mutated cells [78]. Reminiscent of *Atm*-null animals, mutated *IDH1* was associated with a decrease in long-term hematopoietic stem cells (LT-HSCs), which was likely caused by DNA damage-induced differentiation of these cells [79]. Conversely, myeloid-specific mutant IDH1 knock-in mice displayed a significant increase in short-term hematopoietic stem cells, multipotent progenitors, and common myeloid progenitors, presumably due to differentiation defects caused by TET2 inhibition [78]. The impaired DNA damage signaling caused by reduced ATM expression in LT-HSCs might promote the acquisition of additional mutations and clonal expansion of IDH1/2 mutated LT-HSCs. The nature and diversity of such additional hits occurring in LT-HSCs remain to be determined, and would provide crucial information regarding the sequential events leading to mutant IDH1/2-driven transformation.

In aged animals, *Idh1* mutation caused defective DNA repair and increased sensitivity to ionizing radiation and daunorubicin [78]. The *R*-2HG-sensitive lysine demethylases KDM4A and KDM4B orchestrate DNA repair by regulating recruitment of repair factors at DNA double-strand breaks [80,81]. Furthermore, IDH mutations and *R*-2HG, by impeding DNA double-strand break repair via homologous recombination through inhibition of KDM4A/B, increased sensitivity to PARP inhibitors [82], a promising class of anticancer drugs currently in clinical trials. *IDH* mutant cells also displayed enhanced response to monofunctional alkylating agents (e.g. methyl methanesulfonate, MMS [83]), which induce a variety of alkylated DNA bases including 1-methyladenine (1meA) and 3-methylcytosine (3meC). Such lesions can be repaired by the ALKB family of αKG-dependent dioxygenases [84] comprising nine members in human cells (ALKBH1-8 and FTO). The DNA repair activity of mammalian ALKBH2 and ALKBH3, which can reverse alkylation on 1meA and 3meC [85,86], is inhibited by *R*-2HG *in vitro* (Table 1). Moreover, *R*-2HG production sensitizes *IDH* mutant cells to alkylating agents [83]. The clinically important bifunctional alkylating agents procarbazine and CCNU/lomustine, which induce highly genotoxic DNA interstrand crosslinks, are part of the PCV (procarbazine/lomustine/vincristine) chemotherapeutic regimen (in combination with the inhibitor of microtubule assembly vincristine) that is used in conjunction with radiotherapy to treat brain tumors. Importantly, the success of the PCV regimen is associated with *IDH* mutation status because patients carrying such mutations exhibit an overall survival of 9.4 years compared to 5.7 years for patients with wild-type *IDH* [87].

DNA is also susceptible to oxidative damage caused by ROS. Wild-type IDH1/2 catalyze the reduction of $NADP^+$ into NADPH during conversion of isocitrate into α-ketoglutarate. By contrast, mutant IDH1/2 consumes NADPH and α-ketoglutarate to produce *R*-2HG and $NADP^+$ [5,26,33], thus hampering the production of NADPH [88]. NADPH protects against oxidative stress through a variety of mechanisms, including reducing glutathione (GSH), which detoxifies ROS. Thus, decreased NADPH production in *IDH1/2* mutated cancers might increase sensitivity to oxidative stress and DNA damaging agents [89–91]. However, while an altered ratio of $NADP^+$ and NADPH has been observed *in vivo*, increased levels of ROS have not been reported in *Idh* mutant animal models [25,92]. These findings challenge the contribution of ROS to the increased sensitivity to DNA damaging agents of mutated IDH1/2 tumors *in vivo*.

Overall, the downregulation of ATM levels by *R*-2HG might allow IDH1 mutated cells to evade tumor suppression because ATM plays a crucial role in activating p53 and cellular senescence upon oncogenic stress [93–95]. Whether cellular senescence opposes IDH1/2-mediated transformation remains to be determined. In addition, failure to detect and repair DNA damage in IDH1/2 mutant cells could not only contribute to cancer initiation by promoting mutagenesis

but may also increase their sensitivity to genotoxic chemotherapeutics [78,83,90,91]. Accordingly, the increased sensitivity of IDH1/2 mutated tumors to DNA damaging agents might explain the globally positive outcome that is associated with *IDH1/2* mutations in astrocytomas and glioblastomas [14]. Consequently, treatment with pharmacological inhibitors of mutated IDH1/2 may potentially interfere with radiotherapy and chemotherapy in patients carrying such mutations [96].

Control of mTOR Signaling by *R*-2HG

The mammalian/mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that is frequently activated in both IDH1/2 wild-type and mutated brain tumors [97,98]. mTOR senses the presence of mitogenic signals and the availability of nutrients/cellular energy to regulate cell growth, proliferation, autophagy, survival, and metabolism [99]. mTOR forms two kinase complexes, mTORC1 and mTORC2, that respond to different signaling inputs and display distinct substrate specificities. While mTOR, mLST8, and DEPTOR are part of both complexes, mTORC1 specifically includes RAPTOR and PRAS40, whereas RICTOR, mSIN1, and PROTOR1/2 are only found in mTORC2. Upon stimulation with nutrients or mitogenic signals, the mTORC1/2 inhibitory protein DEPTOR, a negative regulator of mTOR, is subject to βTrCP-dependent ubiquitylation and degradation by the proteasome [100–102], thus allowing mTOR activation.

Genomic analysis of GBM revealed that mutations in IDH1/2 and upstream regulators of mTOR signaling pathways, such as PTEN, NF1, and EGFR, are mutually exclusive in that the latter are frequently mutated in IDH1/2 wild-type but not mutant tumors [15,97]. The increased mTORC1/2 activation observed in IDH1/2 mutated brain cancers in the absence of EGFR/NF1/PTEN aberrations suggested an alternative mode of mTOR regulation by *R*-2HG. As such, IDH1/2 mutations and *R*-2HG can stimulate the activation of both mTORC1 and 2 in various cell types (normal human immortalized astrocytes, *Tp53*$^{-/-}$ murine embryo fibroblasts, and HeLa cells), strengthening the notion that *R*-2HG is a metabolite promoting unscheduled activation of signaling pathways involved in cell proliferation and survival [91,97]. *R*-2HG-mediated inhibition of the lysine demethylase KDM4A causes destabilization of DEPTOR protein [97], the endogenous inhibitor of mTORC1/2 [103]. KDM4A interacts with mTORC1/2 through DEPTOR and prevents its ubiquitination by βTrCP [97]. By contrast, *R*-2HG binds to and inhibits the ATP5B subunit of ATP synthase, causing a decrease in ATP/ADP ratio and reduced oxygen consumption [104]. Inhibition of ATP5B by *R*-2HG or expression of IDH1^{R132H} leads to a reduction of mTORC1 signaling in *PTEN*-deficient U87 human glioblastoma cells or in *KRAS* and *PIK3CA* mutated HCT116 colon carcinoma cells [104]. The apparent discrepancies regarding regulation of mTOR by *R*-2HG may stem from the cellular context (i.e., *PTEN*, *PIK3CA*, *KRAS* mutation status) or may suggest context-dependent rewiring of cellular signaling networks, thus allowing diverse effects of *IDH1/2* mutations on mTOR activity. Nonetheless, human patient data show that mutant IDH1/2 cancers exhibit elevated mTOR activity in the absence of genetic alteration in the canonical PTEN/AKT/mTOR pathway [97,105], which supports the role of *R*-2HG in non-canonical activation of mTOR. Activation of mTOR by *R*-2HG is expected to regulate various aspects of IDH1/2-mediated tumorigenesis given the crucial role that this kinase plays in numerous cellular processes [99]. Nevertheless, whether mTOR activation is essential for the transformation of normal astrocytes expressing mutated forms of IDH1/2 remains to be determined.

Modulation of HIF1 Activity by Mutant IDH1/2 and *R*-2HG

Hypoxia-inducible factors (HIFs) 1 and 2 are transcriptional activators that regulate several cellular pathways as a function of oxygen availability [106,107]. In response to low oxygen, HIF signaling contributes to oxygen homeostasis both by promoting angiogenesis and by inducing

a metabolic switch from oxidative phosphorylation to glycolysis to limit oxygen consumption. Key transcriptional targets of HIF also include genes favoring cell proliferation and survival. Given the presence of hypoxic regions in solid tumors, HIF levels are generally increased in numerous types of cancer relative to normal tissue. The resulting elevated HIF activity induces the expression of genes relevant for cancer cell fitness [108]. Moreover, various cancer-associated mutations promote aberrant HIF activation independently of oxygen status [106–108].

HIFs are heterodimers composed of one α subunit (either HIF1α or 2α) and a common 1β subunit (also known as ARNT1). While the β subunit is stable, the α subunit is subject to negative regulation by two types of Fe(II)-dependent dioxygenases: the prolyl hydroxylase domain proteins (EGLN1 and 2, also known as PHD2 and 1, respectively) and the factor inhibiting HIF (FIH, also known as HIF1AN), an asparaginyl hydroxylase. Prolyl hydroxylation of HIFα by EGLNs increases HIFα affinity for VHL ubiquitin ligase, triggering its ubiquitylation and rapid proteasomal degradation [109–111]. Hydroxylation of HIF by FIH blocks association of HIF with the transcriptional coactivators CREB-binding protein (CBP) and p300 [112–114].

Importantly, both EGLN1/2 and FIH require αKG as a co-substrate, suggesting a potential role for IDH1/2 mutations and *R*-2HG production in stimulating HIF activity [115]. Consistently, an initial study aimed at identifying the roles of IDH1 mutation in tumorigenesis described a significant accumulation of the HIF1α paralog in IDH1 mutated gliomas [116]. Several groups subsequently reported conflicting observations in this respect when examining the impact of IDH mutations on HIF1 induction and/or hypoxia gene signatures in gliomas and myeloid cells using cell lines, mouse models, and patient samples. These studies noted increased HIF1 activity or hypoxic response [39,92,117–119], no HIF1 alterations [20,25,120], or diminished HIF1 levels or HIF1-responsive gene levels [121–124]. These discrepancies may reflect the complexity of the modes of regulation controlled by *R*-2HG. One proposed explanation was that, because *R*-2HG can stimulate EGLN1 and EGLN2 activities *in vitro*, the former may blunt HIF1 induction under some conditions [123]. However, this interpretation has been challenged as *R*-2HG only appears to weakly bind to EGLN1 [125]. *R*-2HG rather undergoes non-enzymatic conversion into αKG, which is sufficient to promote EGLN1 activity *in vitro* [125]. Considering that EGLN1 is inefficiently inhibited by *R*-2HG [38], IDH1/2 mutations may not primarily act on HIF1 by modulating EGLN1/2 activity. Instead, *R*-2HG-mediated inhibition of FIH [38,123] could enhance HIF1 signaling.

Endostatin is a secreted anti-angiogenic peptide [126] generated by proteolytic processing of collagen XVIII within the extracellular matrix [127,128]. The HIF1 pathway, among others, is downregulated by endostatin treatment, which markedly reduces *HIF1A* mRNA levels while increasing expression of FIH [129]. Interestingly, endostatin production is enhanced by a collagen-4-prolyl hydroxylase [130], another Fe(II)- and αKG-dependent dioxygenase inhibited by *R*-2HG *in vitro* [123]. Therefore, downregulation of endostatin in cells expressing $IDH1^{R132H}$, and in IDH mutated glioma samples [39], represents a possible mechanism for induction of HIF1.

Thus, *R*-2HG-dependent effects on HIF regulators may depend on the cellular context, level of hypoxia, tumor stage, and anticancer therapy, as well as on extracellular matrix stiffness [124]. Furthermore, because HIF1α and its transcriptional program are stimulated by mTORC1 activation [131], *R*-2HG-mediated control of mTOR activity [97,104] could add a layer of complexity to the regulation of HIF in *IDH1/2* mutated tumors.

Collagen Maturation Defects Triggered by IDH1 Mutation

The basement membrane (BM), separating the epithelium or endothelium from the underlying stroma, provides structural support and regulates cell behavior. Components of the BM are

functionally important for blood vessels and participate in angiogenesis [132]. The BM is composed of numerous proteins, with type IV collagen constituting >50% and allowing the interaction of astrocytes with endothelial cells [133]. All type IV collagen proteins share a similar domain structure, in other words an N-terminal 7S domain, a central triple-helical domain, and a C-terminal globular non-collagenous domain (NC1) [132]. Proline and lysine residues of these proteins are subject to hydroxylation by the αKG-dependent prolyl 4-hydroxylases 1, 2, and 3 (P4HA1/2/3), and procollagen-lysine 2-oxoglutarate 5-dioxygenases 1, 2, and 3 (PLOD1/2/3), respectively. Increased lysine and proline hydroxylation of mature collagen proteins is essential for stability of the triple helix [134]. Accumulation of *R*-2HG in a brain cancer-specific $IDH1^{R132H}$ conditional knock-in mouse embryos led to impaired hydroxylysine-mediated glycosylation of type IV collagen, as well as to its increased solubility, which reflects maturation defects [92]. In addition, defective maturation of collagen caused accumulation of misfolded proteins in the endoplasmic reticulum (ER), triggering an ER stress response [92]. Thus, fragility of the BM caused by mutated IDH1-dependent collagen maturation defects might contribute to brain hemorrhage occurring in $IDH1^{R132H}$ conditional knock-in mice. In the context of IDH1/2 mutated glioma, BM disruption could facilitate tumor progression by allowing epithelial cell invasion, thus favoring angiogenesis [135]. However, the contribution of impaired BM structure during $IDH1^{R132H}$-induced tumorigenesis or disease progression remains to be elucidated. Because the affinity and sensitivity of collagen hydroxylases (P4HA1-3 and PLOD1-3) for *R*-2HG are relatively weak *in vitro* [40], further investigation of the impact of *R*-2HG on collagen hydroxylase activity *in vivo* would provide important molecular clues to the mechanism underlying regulation of collagen maturation by *IDH1* mutations.

Concluding Remarks

The emergence of novel technologies has facilitated the identification of gene mutations associated with metabolic defects in cancer cells. Indeed, it has become evident that alteration of cellular metabolism constitutes a crucial step in tumor development [1]. *IDH1/2* mutations are early events in gliomagenesis [16,17] and leukemogenesis [18,19], and exert effects during tumor initiation through progression. The prognosis for patients with brain tumors such as gliomas and glioblastomas is extremely poor. However, *IDH1/2* mutations are associated with a more favorable outcome in patients afflicted with glioblastoma or anaplastic astrocytoma [14]. Conversely, the prognostic significance of *IDH* mutations in AML remains controversial, although it seems that the overall survival of AML patients carrying wild-type or mutated *IDH1/2* is similar [136]. These differences in the outcome of patients with AML versus brain cancer may suggest a context-dependent response to therapy and imply that different therapeutic regimens are required. Furthermore, the contribution of *IDH1/2* mutations to acquired drug resistance remains unknown.

Although the full impact of the metabolic defects caused by *IDH1/2* mutations is unclear (see Outstanding Questions), these mutations constitute exciting targets in the treatment of numerous malignancies. Small-molecule inhibitors specific for the mutated forms of either IDH1 or 2 are currently under clinical trials in patients with AML, as well as for solid tumors such as cholangiocarcinomas and low-grade gliomas (Figure 1) ([137] for review). Immunotherapy is also being considered as a potential treatment for $IDH1^{R132H}$ gliomas because vaccination with a mutation-specific peptide elicited an antitumor immune response in mice [138]. By contrast, IDH mutation and 2HG production may lead to immune evasion by reducing the expression of chemokines and immunity-related genes [139,140]. Because this effect can be reversed by treatment a specific mutant IDH1 inhibitor, immunotherapy, used in combination with IDH1-targeting drugs, may represent a promising strategy to treat IDH1 mutant gliomas [140]. In addition, *IDH1/2* mutations are associated with increased sensitivity to DNA damaging agents, and might represent biomarkers of treatment efficacy with specific drugs such as PARP1 inhibitors [82]. Overall, a better understanding of the molecular mechanisms promoting

Outstanding Questions

Do metabolic changes induced by *R*-2HG and IDH1/2 mutations (altered NADPH production, increased free amino acids, lower levels of late TCA cycle metabolites, and decreased *N*-acetylated amino acids) contribute to initiation or progression of brain cancer, and by which molecular mechanism(s)?

Because brain-specific $IDH1^{R132H}$ conditional knock-in mice do not develop brain tumors, additional oncogenic events might be required to trigger gliomagenesis. What are the genetic lesions collaborating with *IDH1/2* mutations to induce brain cancer?

Why are some tissues more sensitive to neoplasias triggered by *IDH1/2* mutations?

Does modulation of mTORC1 activity by *R*-2HG contribute to the regulation of HIF1α observed in some IDH mutant tumors, and if so, by which mechanism?

What are the relative sensitivities of different αKG-dependent dioxygenases to *R*-2HG-mediated inhibition?

oncogenesis in the context of *IDH1/2* mutations would allow the identification of potentially more efficient and targeted therapies to treat these malignancies.

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