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Review

The driver and passenger effects of isocitrate dehydrogenase 1 and 2 mutations in oncogenesis and survival prolongation

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ABSTRACT

Mutations in isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) are key events in the development of glioma, acute myeloid leukemia (AML), chondrosarcoma, intrahepatic cholangiocarcinoma (ICC), and angioimmunoblastic T-cell lymphoma. They also cause *D*-2-hydroxyglutaric aciduria and Ollier and Maffucci syndromes. *IDH1/2* mutations are associated with prolonged survival in glioma and in ICC, but not in AML. The reason for this is unknown. In their wild-type forms, IDH1 and IDH2 convert isocitrate and $NADP^+$ to α-ketoglutarate (αKG) and NADPH. Missense mutations in the active sites of these enzymes induce a neo-enzymatic reaction wherein NADPH reduces αKG to *D*-2-hydroxyglutarate (*D*-2HG). The resulting *D*-2HG accumulation leads to hypoxia-inducible factor 1α degradation, and changes in epigenetics and extracellular matrix homeostasis. Such mutations also imply less NADPH production capacity. Each of these effects could play a role in cancer formation. Here, we provide an overview of the literature and discuss which downstream molecular effects are likely to be the drivers of the oncogenic and survival-prolonging properties of *IDH1/2* mutations. We discuss interactions between mutant IDH1/2 inhibitors and conventional therapies. Understanding of the biochemical consequences of *IDH1/2* mutations in oncogenesis and survival prolongation will yield valuable information for rational therapy design: it will tell us which oncogenic processes should be blocked and which "survivalogenic" effects should be retained.

Contents

Abbreviations: AKG, α-ketoglutarate; AML, acute myeloid leukemia; BCAT1, branched-chain amino acid transporter 1; BRD4, bromodomain-containing protein 4; *D*-2HG, *D*-2-hydroxyglutarate or *R*-2-hydroxyglutarate; *D*-2HGA, *D*-2-hydroxyglutarate aciduria; D2HGDH, *D*-2-hydroxyglutarate dehydrogenase; ECM, extracellular matrix; EGLN, egg-laying defective nine; (G)-CIMP, (glioma) CpG island methylator phenotype; G6PDH, glucose-6-phosphate dehydrogenase; GSH, glutathione (reduced); HIF(1α), hypoxia-inducible factor (1α); IDH, isocitrate dehydrogenase; ICC, intrahepatic cholangiocarcinoma; IR, ionizing radiation; JHKDMs, Jumonji-C domain-containing histone lysine demethylases; JMJC, Jumonji-C; KDM, lysine demethylase; *L*-2HGM, *L*-2-hydroxyglutarate or *S*-2-hydroxyglutarate; *L*-2HGA, *L*-2-hydroxyglutarate aciduria; MDS, myelodysplastic syndrome; MGMT, O^6-methylguanine-DNA methyltransferase; MPN, myeloproliferative neoplasms; ROS, reactive oxygen species; SNP, singlenucleotide polymorphism; TET2, ten–eleven translocation 2; TMZ, temozolomide; VEGF, vascular endothelial growth factor

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1. Introduction

Over the last decade, genome-wide sequencing of cancers has uncovered novel causative alterations and possible therapeutic targets. This led to the discovery of mutations in isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2)* in various cancers and genetic (metabolic) diseases [1–13]. These mutations attracted considerable interest due to the resurgence of intracellular metabolic reprogramming in cancer research [14] and the potential consequences of the neo-enzymatic conversion of α-ketoglutarate (αKG) to D-2-hydroxyglutarate (*D*-2HG) (Fig. 1) [15]. The accumulating *D*-2HG competitively inhibits αKG-dependent enzymes, causing cellular alterations in epigenetics, collagen maturation, and hypoxia signaling [16–22].

Another aspect of *IDH1* mutations is the association with a relatively prolonged patient survival for glioblastoma [1] and intrahepatic cholangiocarcinoma (ICC) [5] but not for acute myeloid leukemia (AML) [23]. It remains to be determined whether *IDH1* mutations are causative for the prolonged survival in glioblastoma and ICC. If so, there could be intrinsic differences in cancers mediated by *IDH1/2* mutations or the differences could be due to differential interactions with therapies. Both possibilities are reviewed.

Patients will soon be enrolled in clinical trials of mutant IDH1/2 inhibitors [24,25]. A reasonable concern is that such drugs may eliminate the putative survival prolonging-effects of *IDH1/2* mutations. Therefore, it is important to identify effects that drive oncogenesis and thus need to be blocked and effects that prolong patient survival and should thus be retained.

This review discusses current understanding of *IDH1/2* mutations and their role in oncogenesis and prolonged survival. *IDH1/2* mutations result in inhibition of more than 60 αKG-dependent dioxygenases, many of which may not even be relevant to oncogenesis or prolonging patient survival. In addition, lack of one wild-type IDH1/2 implies less NADPH production, the net result being less capacity to protect cells against oxidative stress. This review discusses which downstream effects of *IDH1/2*-mutations are the drivers of cancer initiation/progression and/or prolonging patient survival as opposed to mere passenger effects.

2. Normal function of IDH1/2 enzymes

IDH1 and IDH2 are homodimeric enzymes that reversibly convert isocitrate to αKG in the cytoplasm and mitochondria, respectively, presumably for the purpose of the concomitant reduction of $NADP^+$ to NADPH (Fig. 1,2) [26]. NADPH is an important source of synthetic reducing power and has key functions in cellular detoxification processes, because it is necessary for the reduction of glutathione (GSH) [27] and thioredoxins [28], the formation of active catalase tetramers [29] and activity of cytochrome P450 [27,30]. These are all involved in the reduction of reactive oxygen species (ROS). αKG also possesses detoxifying properties [31–33]: it reduces H_2O_2 to water as it decarboxylates to succinate [33]. In most human tissues, particularly the brain, IDH1 is the most important NADPH producer [34,35]. This contrasts the situation in myeloid cells, where glucose-6-phosphate dehydrogenase (G6PDH) of the pentose phosphate pathway is the major NADPH provider [36].

In hypoxic contexts, IDH1 and IDH2 can also convert αKG to isocitrate. This reductive carboxylation is the reverse of the normal, oxidative decarboxylation. Hypoxia inhibits pyruvate production and therefore TCA cycle flux via upregulation of hypoxia-inducible factor 1α (HIF1α; Fig. 1). In such contexts, cells use glutaminolysis to produce citrate, which can be used in fatty acid synthesis (serving as an acetyl-CoA shuttle) or NADPH production (via IDH1 in decarboxylation mode). The conversion of αKG to isocitrate by IDH1/2 is part of this "extended" glutaminolysis in mitochondria [37–40].

The IDH enzyme family also includes TCA cycle members IDH3A, B and C that are structurally divergent heterotetrameric NAD^+-dependent dehydrogenases. Mutations in IDH3A, B and C in relation to oncogenesis have not been described.

3. *IDH1/2* mutations in cancer

3.1. Glioma

Somatic mutations in *IDH1* and *IDH2* occur frequently (50–80%) in adult glioma (WHO grade II and III) and secondary glioblastoma

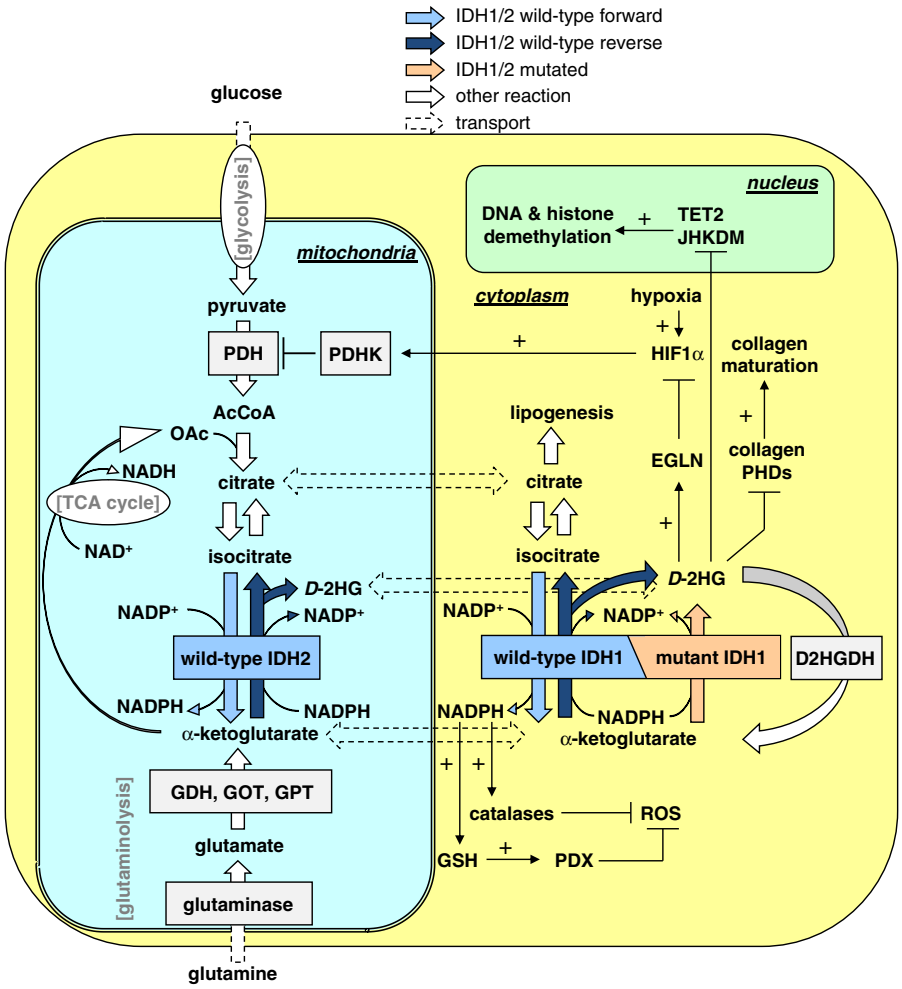

Fig. 1. Molecular and cellular effects of mutant IDH1 in a cell and its components. Wild-type IDH1 and IDH2 (blue) catalyze the reversible conversion of isocitrate to αKG, with the concomitant production of NADPH from $NADP^+$. Mutant IDH1 and IDH2 (orange) convert α-ketoglutarate to *D*-2HG while reducing NADPH to $NADP^+$, leading to a decreased NADPH production capacity. *D*-2HG increases DNA and histone methylation via TET2 and JHDM inhibition, impairs collagen maturation via collagen PHD inhibition and promotes HIF1α degradation via EGLN activation. Abbreviations: PDH, pyruvate dehydrogenase; PDHK, pyruvate dehydrogenase kinase; AcCoA, acetyl coenzyme A; OAc, oxaloacetate; PHD, prolyl hydroxylase; GDH, glutamate dehydrogenase; GOT, glutamate oxaloacetate transferase; GPT, glutamate pyruvate transferase; PDX, peroxiredoxin.

(WHO grade IV; Table 1) [1,2,41–45]. In contrast, they are rare or even non-existent in primary glioblastoma, in WHO grade I pilocytic astrocytoma, in glioma in children and the elderly and other types of brain tumors [41,46,47]. Patients with *IDH1/2*-mutated glioma are on average younger (~30 years vs ~60 years) and have a relatively favorable prognosis over their counterparts with wild-type *IDH1/2* glioma (median survival 31 months vs 12 months in glioblastoma [1]). In a whole-exome sequencing effort of low-grade glioma and their recurrences, *IDH1* mutations were the only persisting mutations in all patients. This implicates *IDH1* mutations as initiating events in low-grade gliomagenesis and as promising therapeutic targets [43,48,49].

IDH1/2 mutations are located within the enzymatically active sites at amino acid residues 100 or 132 (IDH1) and 140 or 172 (IDH2). The vast majority of *IDH1/2* mutations in glioma are *IDH1* mutations and of these, 90% are $IDH1^{R132H}$ (Table 1), while R132C, R132G, R132L and R132S occur at lower frequencies. Equivalent mutations, R172K, R172M and R172W, affect the catalytic site of IDH2 [2,41,45]. In addition, rare $IDH1^{R100Q}$ mutations have been described in four WHO grade II and III glioma, also affecting the catalytic site [50,51]. Of note, SNPs at 8q24.21 and 11q23 have been described to be strongly associated with *IDH1/2*-mutated glioma [69,70].

3.2. Myeloid hematological disorders

IDH1/2 mutations occur in 15% of *de novo* normal-karyotype AML and 20% of AML that has progressed from myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN) (Table 1) [23,52–54]. In contrast with glioma, *IDH2* mutations occur more frequently (~60%) than *IDH1* mutations (~40%) in AML, with $IDH2^{R140Q}$ as the most common mutation [23,54]. In AML, *IDH1* mutations correlate with worse patient survival, whereas $IDH2^{R140Q}$ mutations are associated with a moderately prolonged survival [55,56]. In addition, *IDH1/2* mutations are associated with an unfavorable prognosis in MDS and MPN. The frequency of *IDH1/2* mutations increases with disease stage; low (5%) in early stage MDS and MPN and high (20%) in late-stage disease and secondary AML [53,57,58], suggesting that *IDH1/2* mutations promote malignant transformation to full-blown AML.

3.3. Other types of cancer and syndromes

IDH1/2 mutations occur in varying proportions in other types of cancers: angioimmunoblastic T-cell lymphoma [8]; acute lymphocytic leukemia [12]; chondrosarcoma (CS) [9,11]; ICC [4–7]; rare cases of colon

Table 1
Mutation frequencies of *IDH1/2* in different types of cancer.

Cancer type	Preferred subtype or location for *IDH* mutations	*IDH1/2* mutated (%)	% *IDH1* mutations	Mutant alleles	Mutant frequency (% of total)
ALL		5–10	50	$IDH1^{R132H}$	30
				$IDH1^{R132G/S}$	20
				IDH2 unknown	50
AML	Normal karyotype AML, secondary AML	10–40	30–50	$IDH1^{R132H}$	10–20
				$IDH1^{R132}$ other	15–30
				$IDH1^{R100Q, R104V, F108L, R119Q, I130V}$	<5
				$IDH2^{R140Q}$	30–50
				$IDH2^{R140}$ other	5
				$IDH2^{R172}$	10–15
Cholangiocarcinoma	Intrahepatic cholangiocarcinoma	10–20	80	$IDH1^{R132C}$	50
				$IDH1^{R132L/S/V}$	30
				$IDH2^{R172K/S/W}$	20
Chondrosarcoma	Central, periosteal and dedifferentiated chondrosarcoma	50–70	95	$IDH1^{R132C}$	50
				$IDH1^{R132}$ other	30–40
				$IDH2^{R172S/T}$	10
Gastric cancer	Early gastric cancer	7	0	$IDH2^{G145R}$	100
Glioma	WHO grade II, III glioma, secondary glioblastoma	60–90	95	$IDH1^{R132H}$	90
				$IDH1^{R132}$ other	5
				$IDH1^{R100Q}$	<1
				$IDH2^{R172}$	5
Osteosarcoma		30	0	$IDH2^{R172S}$	100
T-cell lymphoma	Angioimmunoblastic T-cell lymphoma	10–40	0	$IDH2^{R172K}$	75
				$IDH2^{R172G/M/S/T}$	20–25
				$IDH2^{R140G}$	5

cancer [59]; gastric cancer [60]; medulloblastoma [47]; melanoma [61]; non-small cell lung cancer [62]; osteosarcoma [63]; paraganglioma [64]; and prostate cancer [12]. In addition, *IDH1/2* mutations are causative in Ollier disease, Maffucci syndrome [10,65] and *D*-2-hydroxyglutaric aciduria (*D*-2HGA) [13,66].

In Ollier disease and Maffucci syndrome [10,65], patients develop multiple cartilaginous tumors (enchondroma) during childhood and also glioma are described in both syndromes. Only in Maffucci syndrome, hemangioma have been described [67]. The enchondroma and glioma of these patients are *IDH1/2* mutated, whereas wild-type IDH1/2 is expressed in most healthy tissue. This has led to the hypothesis that *IDH1/2* mutations occur during embryonic development in a somatic mosaic pattern and that the IDH1/2-mutant tumors are derived from these *IDH1/2*-mutated cells [10].

D-2HGA is an inherited metabolic disease that is caused by $IDH2^{R140Q}$ mutations in 50% of cases and is characterized by developmental delay, hypotonia, seizures and cardiomyopathy with an onset in the first year of life. Patients often decease in the first decade [68]. The cardiac and neurodegenerative symptoms are directly caused by *D*-2HG accumulation, although the exact mechanisms remain unclear [69]. Another metabolic inherited disease is *L*-2-hydroxyglutaric aciduria (*L*-2HGA), in which *L*-2HG accumulates. *L*-2HGA is a clinically milder disease than *D*-2HGA. Of note, *L*-2HGA patients have an increased incidence of glioma, but no other tumors [68]. No such predisposition for gliomagenesis has been described in *D*-2HGA, but since *D*-2HGA is lethal at younger ages than *L*-2HGA, *D*-2HGA patients may not live long enough to develop glioma.

4. Neoenzymatic function of mutant IDH1/2

4.1. Mutant IDH1/2 produces D-2HG

Heterozygous *IDH1* mutations in glioblastoma result in the loss of one wild-type IDH1 copy and decrease the IDH1-facilitated NADPH production capacity by 50% [34,35]. Moreover, mutant IDH1/2 causes a loss of function through a dominant-negative inhibition of wild-type IDH1/2 [2,70].

In addition, IDH1/2 mutations confer a neoenzymatic gain of function. In the catalytically active site, replacement of the strong-positively charged Arg with amino acids that have lower electrical polarities, such as His, Lys, Gln or Cys impedes the formation of hydrogen bonds with the α- and β-carboxyl domains of isocitrate [70]. As a result, the binding affinity is decreased for isocitrate and increased for NADPH. This initiates a reaction that is similar to the reverse wild-type reaction (wherein αKG is reductively carboxylated to isocitrate) but differs in that αKG is reduced but not carboxylated. Thus, the reaction yields 5-carbon *D*-2HG rather than 6-carbon isocitrate and oxidizes NADPH to $NADP^+$ [15]. All IDH1/2 mutations studied have this gain of function [15,71,72] and exclusively produce the *D*- (or *R*-) enantiomeric isoform of 2HG, and not the *L*- (or *S*-) enantiomer (Fig. 2) [15]. Until now, there have been no reports of *L*-2HG-producing *IDH1/2* mutations, neither naturally occurring, nor laboratory-constructed.

The accumulation of *D*-2HG may be utilized diagnostically in oncology clinics. *D*-2HG in serum and urine correlates with AML and ICC disease recurrence and can be used to determine the *IDH1/2* mutation status [72–77]. Accordingly, proton or ^{13}C magnetic resonance microscopy can detect intracranial *D*-2HG accumulation in *IDH1/2*-mutated glioma [78–81].

4.2. IDH1/2 mutants differ in D-2HG production

The different *IDH1/2* mutants ($IDH1^{R132}$, $IDH2^{R140}$, $IDH2^{R172}$ and their variants) possess varying enzymatic properties. The most common IDH1/2 mutants in glioma and AML ($IDH1^{R132H}$ and $IDH2^{R140Q}$, respectively) are weak *D*-2HG producers, as compared with other variants. For example, the most common IDH1/2 mutant in CS ($IDH1^{R132C}$), produces relatively high levels of *D*-2HG [71,82–84]. The only IDH1/2 mutant that is found in the inherited metabolic disease *D*-2HG aciduria is the weakest *D*-2HG-producing $IDH2^{R140Q}$ mutant. It has been proposed that only the lower *D*-2HG levels that result from such mutations are compatible with embryogenesis; *IDH1/2* germline mutations leading to higher *D*-2HG levels may be embryonically lethal [83]. It has been hypothesized that in oncogenesis, the intracellular *D*-2HG concentration that gives the largest growth advantage varies depending on the tumor's cell type of origin. This could explain why each type of cancer has a specific favorite *IDH1/2* mutation. In addition, an *IDH1/2* mutation and the subsequent high *D*-2HG levels may affect which specific type of cancer is being formed [85].

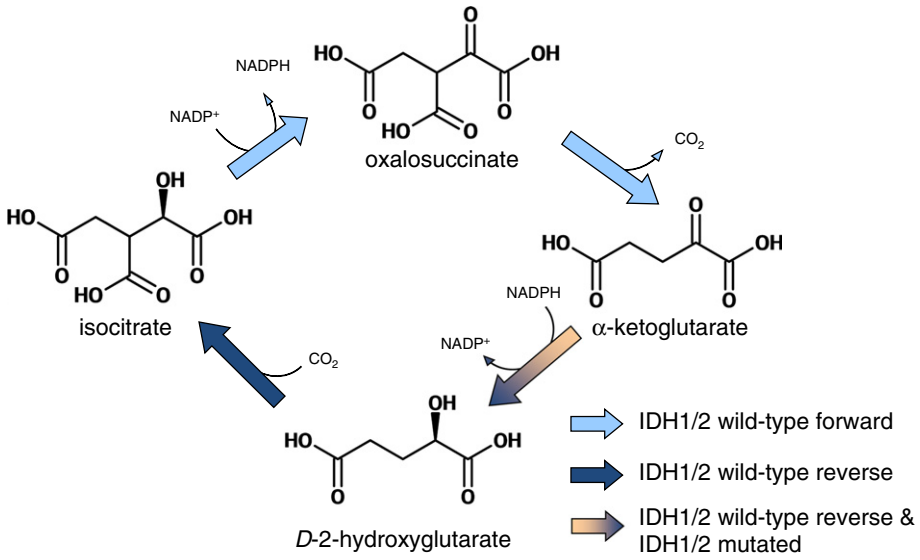

Fig. 2. Chemical effects of wild-type and mutant IDH1/2. Wild-type IDH1/2 enzymes (area covered in blue) catalyze a two-step reaction in which isocitrate is first oxidized by $NADP^+$ to form the intermediate oxalosuccinate and NADPH. Subsequently, IDH1/2 decarboxylyzes oxalosuccinate to αKG and CO_2. Mutant IDH enzymes possess a neoenzymatic function (area covered in orange): they catalyze a reaction in which αKG is reduced to *D*-2HG. Here, NADPH acts as a hydrogen donor and is oxidized to $NADP^+$.

A great variety of IDH1/2 mutants can be conceived, spanning a comprehensive spectrum of *D*-2HG accumulation levels [83,84,86]. Thus, optimal *D*-2HG concentrations alone do not satisfactorily explain why *IDH1* mutations are much more prevalent than *IDH2* mutations in glioma, ICC and CS. Another reason may be that in hypoxia, IDH1/2 can operate in reverse to use glutaminolysis to produce citrate, which can subsequently be used for fatty acid production. In vitro studies have shown that different cell lines use either IDH1 or IDH2 or both for these reversed reactions. SF188 glioblastoma cells use IDH2 to catalyze reductive carboxylations [39], A549 lung carcinoma, HCT116 colon carcinoma, MF10A mamma epithelial and MDA-MB-231 mamma carcinoma cells use IDH1 [38,40] and 143B osteosarcoma cells can use both IDH1 and IDH2 [37]. The reason why some cells prefer IDH1 or IDH2 over the other for reductive carboxylation is unknown at this moment.

Mutations inactivate the capacity of IDH1/2 to carry out their wild-type forward reactions and reverse reactions [26]. Thus, *IDH2* mutations may hinder glioblastoma citrate production, which is essential for cellular proliferation. Although both *IDH1* and *IDH2* mutations promote oncogenesis, only *IDH1* mutations may retain the glioblastoma cells' ability to synthesize critical macromolecules from glutaminolysis. This model captures a selective pressure for *IDH1* mutations over *IDH2* mutations in glioblastoma. Because of the balanced frequencies of *IDH1* and *IDH2* mutations in AML, we speculate that leukemic cells may be able to use both IDH1 and IDH2 for reductive carboxylation.

5. Oncogenic effects of *IDH1/2* mutations

Because *IDH1/2* mutations were described as inaugural events in gliomagenesis [43] and occur in premalignant myeloid disorders [57], *IDH1/2* mutations were suspected to be tumorigenic. Direct evidence for oncogenic properties of *IDH1/2* mutations was presented by retroviral-mediated introduction of $IDH2^{R140Q}$ or $IDH2^{R172K}$ into 10T1/2 mesenchymal progenitor cells, which induced the formation of sarcoma-like tumors [87] and AML [88–90] in mice. Critically, $IDH2^{R140Q}$ was shown to be necessary for AML maintenance in mice [89], indicating that *IDH1/2* mutations may be seen as therapeutic targets in *IDH1/2*-mutated cancers. Also, it may be important to identify the downstream oncogenic drivers of *IDH1/2* mutations to facilitate rational drug design that targets the IDH1/2 mutant pathway.

5.1. Interference with αKG-dependent dioxygenases

The consensus mechanism of *IDH1/2* mutation-induced oncogenesis is that *D*-2HG competitively inhibits or activates αKG-dependent dioxygenases [16,17,19], which are involved in many cellular processes. Four types of affected αKG-dependent dioxygenases and their putative roles in oncogenesis are discussed here: EGLN, TET2, JMJC and collagen hydroxylase. The oncogenic properties of *D*-2HG are supported by the finding that 2HG accumulation also occurs in *IDH1/2* wild-type breast cancers and correlates with a worse prognosis in these tumors [91].

5.1.1. Induction of HIF1α degradation by EGLN prolyl-4-hydroxylases

D-2HG accumulation promotes degradation of HIFs. This occurs via promotion of egg-laying defective nine (EGLN, also called prolyl hydroxylase domain 2 [PHD2]), prolyl-4-hydroxylase activity [18,92], although the mechanism is not completely clear yet [93]. HIF transcription factors mediate responses to hypoxia. EGLN hydroxylates HIF Pro residues that ultimately lead to the proteosomal degradation of HIFs [94]. Accordingly, the expression of 87 HIF1α-responsive genes was markedly decreased in *IDH1*-mutated glioma samples [95].

Whereas an early effect of $IDH1^{R132H}$ is an increase in HIF1α levels [70], HIF1α levels are suppressed at 8–15 passages after $IDH1^{R132H}$ transfection in vitro [18]. We speculate that the initial HIF1α stabilization is explained by a rapid decrease in αKG levels (due to loss of IDH1/2 wild-type function and resulting in EGLN inhibition), whereas later HIF1α degradation may be due to the then-accumulated *D*-2HG that outweighs the lower concentrations of αKG and promotes EGLN activity.

In cancer, HIF expression is upregulated in hypoxic conditions and promotes expression of genes involved in all hallmarks of cancer [14,96], indicating an oncogenic role. However, there is mounting evidence that HIF1α is a tumor suppressor in leukemia [97–99]. In line with this, people living at high altitude and thus residing in a state of chronic hypoxia, resulting in presumably higher levels of HIFs, have a lower risk of developing leukemia [92]. *IDH1/2* mutations may contribute to leukemogenesis by destabilization of HIF1α.

In addition, in vitro and in vivo studies indicate that HIFs have tumor-suppressive functions in glioma as well. In vitro overexpression of EGLN1 or depletion of HIF1α is sufficient to promote soft agar colony

formation of immortalized human astrocytes, whereas EGLN1 knockdown inhibits IDH1-mutant colony growth [18]. In addition, EGLN attenuates HIF2α levels in human astrocytes [18], another tumor suppressor [100]. In vivo glioma studies have shown that the function of HIF1α is context-dependent [101]. HIF1α possesses oncogenic properties when transformed murine astrocytes or human glioma cell lines (U87 and D54) are grown subcutaneously [101–103], but exhibits tumor-suppressive effects when transformed murine astrocytes are grown intracranially [101].

Before *IDH1/2* mutations were discovered, HIF1α was found to be overexpressed in human glioma when compared with normal brain and expression levels correlated with tumor grade [104]. Moreover, HIF1α levels are increased in *IDH1*-mutated glioblastoma as compared with normal brain [70]. Mechanisms that upregulate HIF1α expression in glioblastoma, activation of the RTK/RAS/PI(3)K pathway and inactivation of the p53 pathway, occur in 87% and 88% of glioblastoma, respectively [105]. We speculate that *IDH1/2* mutations promote glioma formation through initial attenuation of HIF1α levels. Subsequently, HIF1α levels are upregulated as a response to hypoxia that is the result of the increased growth of the tumor and a declined oxygen perfusion in areas that are too distant from blood vessels. The increased (but still attenuated by *D*-2HG) HIF1α levels facilitate angiogenic and glycolytic responses to hypoxia. Indeed, HIF1α upregulation in *IDH1/2*-mutated tumors is restricted to areas of presumed severe hypoxia [106]. In addition, the described genetic mechanisms can constitutively activate HIF1α expression.

Necrosis is one of the histological hallmarks of glioblastoma [107]. Necrosis is the result of hypoxia-mediated activation of the coagulation system, which causes intravascular thrombosis. This further increases intratumoral hypoxia and leads to abnormal proliferation of endothelial cells and tumor necrosis [108]. Remarkably, secondary glioblastoma display much lower rates of necrosis than primary glioblastoma [108]. As described in Section 3.1, *IDH1/2* mutations occur very frequently in secondary glioblastoma but are rare in primary glioblastoma [1,2,45]. In accordance with activation of EGLN by *D*-2HG, HIF is markedly decreased in secondary versus primary glioblastoma [109,110]. This may be the result of less hypoxia-mediated activation of the coagulation system in secondary glioblastoma than primary glioblastoma because the frequently occurring *IDH1/2* mutations suppress HIF1α levels and hypoxia responses in secondary glioblastoma.

All in all, we consider degradation of HIF1α as a likely driver of oncogenesis in the context of *IDH1/2* mutations. It remains to be investigated whether the HIF1α signaling axis represents a potential therapeutic target for *IDH1/2*-mutated cancers.

5.1.1.1. Physiological function of the D-2HG-HIF1α signaling axis. As described in Section 2, IDH1/2 catalyze reversed physiological reactions in hypoxic contexts which do not only produce isocitrate, but also yield *D*-2HG. It has been proposed that *D*-2HG regulates these reversed reactions by altering epigenetics of genes that signal for glutamine metabolism and/or hypoxia [111]. We propose that *D*-2HG functions physiologically in a direct negative feedback loop, wherein HIF1α promotes reverse TCA cycle reactions that lead to *D*-2HG production, which subsequently suppresses HIF1α levels by promoting EGLN activity (Fig. 1). Evolution may have taken a risk involving *D*-2HG in this TCA cycle-regulating *D*-2HG-HIF1α signaling axis. Cells occasionally lose this gamble since IDH1/2 structures are now closer to being mutable to forms that overproduce *D*-2HG, derail the *D*-2HG-HIF1α signaling axis and cause cancer.

5.1.2. Epigenetic effects

5.1.2.1. Global hypermethylation by TET2 demethylases. Ten–eleven translocation 2 (TET2) is a member of the TET family of αKG-dependent DNA-modifying enzymes that may be a pathologically relevant target of *D*-2HG (reviewed in [20,112]). *D*-2HG inhibits TET2 activity in vitro [17,18]. TET2 hydroxylates 5-methylcytosine to 5-hydroxymethylcytosine, which mediates DNA demethylation [19].

When TET2-mediated DNA demethylation is inhibited by *D*-2HG, this should lead to DNA hypermethylation. Indeed, *IDH1/2*-mutated AML, glioma, ICC and CS are strongly correlated with global DNA hypermethylation. This could induce oncogenesis by repression of differentiation genes and induction of the expression of stem cell maintenance genes [19,111]. Indeed, mutant *IDH1*-induced DNA methylation blocks cellular differentiation in vitro [22]. In addition, it has been described that cyclin-dependent kinases and genes in the retinoic acid receptor tumor suppressor pathway are methylated or downregulated after introduction of an *IDH1* mutation in vitro [113–115].

Somatic inactivating mutations in *TET2* are common in myeloid hematological disorders, occurring in 50–60% of secondary AML, MDS and MPN [112,116–119]. *TET2* mutations are mutually exclusive to *IDH1/2* mutations in AML [19,119], suggesting that these genes have a similar function. *TET2* mutations are not observed in glioma [120] and are very rare in CS [11], although *TET2* is silenced by *TET2* promoter methylation in 14% of *IDH1/2* wild-type glioma [120].

In vitro studies have shown that *D*-2HG inhibits TET2 [17]. In addition, there is mechanistic evidence that TET2 inhibition contributes to oncogenesis. In a human erythroleukemia cell line (TF-1), TET2 knockdown increased proliferation and blocked cellular differentiation [92]. Administration of 5-azacytidine or decitabine, two demethylating agents, reduces DNA methylation, induces differentiation and inhibits growth of *IDH1*-mutated glioma cells. However, decitabine was aspecific for *IDH1*-mutated cells and the specificity of 5-azacytidine has not been investigated yet [121,122].

5.1.2.2. Histone modifications by JMJC histone demethylases. *D*-2HG also inhibits αKG-dependent Jumonji-C (JMJC) domain-containing histone lysine demethylases (JHKDMs) [16,17,22]. These are considered as tumor suppressors and have been associated with oncogenesis in various types of cancer [123]. JHKDMs modify chromatin to regulate gene expression epigenetically. In non-cancerous cells, tumor-suppressor genes may be part of the transcriptionally active euchromatin and oncogenes may be part of the transcriptionally silent heterochromatin. Inhibition of JHKDMs by *D*-2HG may disturb this balance. Expression of KDM2B and KDM3B is decreased in glioblastoma [124] and AML [125], as compared with healthy tissue. This suggests that (JH)KDM inhibition may have oncogenic functions in cancer.

Introduction of mutant IDH1/2 or *D*-2HG increases lysine histone methylation in HEK293T cells and blocks cell differentiation in central nervous system-derived NHA cells in vitro [22]. However, it is unclear whether inhibition of JHKDMs by *D*-2HG results in epigenetically relevant changes in vivo; some methylation markers were increased in $IDH1^{R132H}$ knock-in mice (H3K79me2, H3K36me3, H3K4me3), whereas others were unchanged (H3K27me3, H3K9me3) [21,88,126]. This suggests that the inhibitory effect of *D*-2HG on JHKDMs may be insufficient to induce methylation changes at some histones. The observed *D*-2HG levels may not significantly alter histone methylation in cancer in vivo, or specifically target biologically important loci that promote tumor formation, but are not broad enough to establish differences in global histone methylation. Whole-genome methylation studies of *IDH1/2*-mutant cancers may address these questions.

5.1.2.3. Epigenetic effects: drivers or passengers?. Accumulating evidence implies the epigenetic effects of *IDH1/2* mutations in malignant transformation. However, there are several arguments against a critical role of epigenetics in oncogenesis in *IDH1/2*-mutated cancers as well. First, all IDH1/2 mutants that are described thus far exclusively produce *D*-2HG, whereas *L*-2HG is a more potent inhibitor of TET2 and JHKDMs [16,17,30]. This contrasts with EGLN, where *D*-2HG promotes EGLN activity and thereby proliferation, whereas *L*-2HG inhibits EGLN activity. The selectivity of IDH1/2 mutants for *D*-2HG production could challenge the assumption that epigenetics have a critical role in oncogenesis. A

counterargument may be that *IDH1/2* mutations that produce *L*-2HG levels sufficient for inhibition of αKG-dependent dioxygenases are simply not realizable.

Second, the results of recent studies suggest that the epigenetic effects of *D*-2HG are of limited relevance for maintenance of glioma cell proliferation. For example, treatment of *IDH1*-mutated TS603 oligodendroglioma cells with a low dose of an $IDH1^{R132H}$ inhibitor showed that a maximum inhibitory effect on proliferation was not accompanied by changes in global DNA methylation, histone methylation and RNA expression levels. A higher dose of the $IDH1^{R132H}$ inhibitor induced expression of differentiation markers and reversed histone methylation (and did not affect DNA methylation (G-CIMP)), but did not achieve additional tumor growth inhibition. Thus, $IDH1^{R132H}$ inhibitors achieve maximal *IDH1*-mutated glioma growth inhibition without altering epigenetics [24]. Although this does not preclude epigenetic effects or CIMP as initial drivers of malignant transformation, it suggests that epigenetics may not represent a therapeutic target for *IDH1/2*-mutated cancers. This is further supported by the finding that the leukemogenic effects of *D*-2HG are reversible within short periods of time after *D*-2HG withdrawal in vitro, whereas epigenetic changes usually take more time. This suggests that either the epigenetic effects of mutant IDH1/2 are surprisingly dynamic or epigenetics are not involved in leukemia maintenance [92].

Third, although knock-down of TET2 in TF-1 cells induces proliferation, concomitant knock-down of EGLN completely abolishes proliferation [92]. Thus, TET2 inhibition needs EGLN activity to promote proliferation. In contrast: HIF1α knock-down or EGLN expression induces proliferation of astrocytes independently of TET2 inhibition [18]. However, these data do not rule out an additive effect of both TET2 inhibition and EGLN activation in oncogenesis.

Fourth, there are irreconcilable phenotypic differences between *IDH1*/2 and *TET2*-mutated tumors. As described earlier, *IDH1/2* mutations are strongly associated with global DNA hypermethylation in all *IDH1/2*-mutated cancers examined [19,22,127,128]. In contrast, *TET2* mutations are associated with hypermethylated [19], unchanged [129] and even hypomethylated DNA genotypes [130,131] in AML patients. Furthermore, *TET2* mutations are associated with a significantly worsened prognosis in AML, with lower complete remission rates [132]. In addition, *TET2* mutations mostly occur in MDS/MPN, whereas *IDH1/2* mutations most are often observed in full-blown leukemia [112]. These phenotypic differences between *IDH1/2*-mutated and *TET2*-mutated leukemias suggest that these enzymes have differential molecular effects and that TET2 is at least not the only driver of oncogenesis.

In conclusion, arguments exist against TET2-mediated and JHKDM-mediated epigenetic modifications being drivers of *IDH1/2* mutation-induced oncogenesis. Reversing global DNA hypermethylation/CIMP may be unattainable and in vitro experiments show that epigenetic changes may not be necessary to inhibit *IDH1/2*-mutated tumor growth [24]. Further research should define whether *IDH1/2*-mutated mutant tumors are sensitive to therapeutic targeting of their modulated epigenetic landscapes.

5.1.3. ECM disturbances by collagen hydroxylases and matrix metalloproteinases

D-2HG inhibits three types of collagen hydroxylases; the PLOD, Leprecan and P4HA families of prolyl hydroxylases, which are required for proper collagen protein maturation [18,21]. Matured collagen is an important component of the extracellular matrix (ECM), which is heavily involved in cellular proliferation and differentiation [133]. Thus, perturbations in the ECM homeostasis may stimulate oncogenesis. In addition, $IDH2^{R172G}$ mutations have been shown to upregulate expression of MMP2 and pro-MMP9, matrix metalloproteinases that degrade the ECM [134,135].

In addition to proliferation, ECM perturbations can also lead to increased invasiveness. It has been proposed that *IDH1/2* mutations and their impact on the tumor microenvironment may explain the diffuse nature of glioma [111]. However, a big caveat is the absence of collagen in normal brain parenchyma [136]. Furthermore, wild-type *IDH1/2* glio(blasto)ma also have a diffuse infiltrating capacity [107]. ECM disturbances may also be hard to reconcile with the formation of hematopoietic cancers, because one would not expect the ECM to be an important factor in the development of leukemia. In contrast, the collagen gene *COL2A1* is frequently mutated in chrondrosarcoma, suggesting that ECM disturbances function in the development of such cartilaginous tumors [11]. With the limited number of studies currently available, we cannot conclude yet whether ECM disturbances by *IDH1/2* mutations drive oncogenesis or not.

5.2. Increased intracellular ROS levels

In glioblastoma, *IDH1* mutations decrease the production capacity of NADPH [34,35], a critical component for cellular detoxification processes. Cells use NADPH to keep glutathione in reduced form (GSH) as well as thioredoxin. In turn, peroxidase and peroxiredoxin use GSH to reduce ROS (Fig. 1). In addition, *D*-2HG also induces oxidative stress directly [137–139] although the mechanisms are unclear. As described, *IDH1/2* mutations induce loss of function through a dominant-negative inhibition of wild-type IDH1/2 [2,70]. This has been attributed to structural changes of wild-type IDH1/2 due to heterodimerization with mutant IDH1/2 [140]. We speculate that *D*-2HG inhibits wild-type IDH1/2 because it competes with isocitrate due to αKG mimicry, similar to *D*-2HG's competitive inhibition of αKG-dependent dioxygenases such as TET2 and JMJC.

ROS create 8-oxoguanine lesions in DNA that can lead to mutations that promote tumor development and progression, such as cellular proliferation, evasion of apoptosis or anoikis, angiogenesis, tissue invasion and metastasis [141]. This has also been observed in neural stem cells [142]. It is possible that *IDH1/2* mutations lead to increased levels of ROS and thus mutator states that promote malignant transformation. For example, in chronic myeloid leukemia, the hallmark Philadelphia chromosome (BCR-ABL) induces ROS formation and transforms the cancer to an AML-like blast phase [143].

IDH1 mutations induce mitochondrial dysfunction and increase the number of mitochondria [137–139,144], which are the primary source of ROS. Overexpression of mutant IDH1 results in increased ROS levels in hematopoietic cells [115]. In vitro and in vivo studies have shown that *IDH1/2* mutations do not alter ROS levels in immortalized human astrocytes [18] or in brains and hematopoietic cells of $IDH1^{R132H}$ knock-in mice, as compared with wild-type mice [21,126]. However, these studies used a ROS marker (CM-H_2DCFDA) that is insensitive to H_2O_2 [115]. On the basis of NADPH consumption by peroxidase and peroxidredoxin reactions, one would expect H_2O_2 levels to be elevated in a cell depleted of NADPH. Increased ROS as a driver of malignant transformation in *IDH1/2*-mutated cancers is very interesting, but mechanistic evidence is required to come to definitive conclusions.

6. Survival-prolonging effects of *IDH1/2* mutations

Glioma carrying *IDH1/2* mutations are associated with prolonged patient survival, as compared with their *IDH1/2* wild-type counterparts, independent of age or clinical performance status [35,44]. *IDH1*/2 mutations are also associated with an improved outcome in ICC patients [5]. In contrast, *IDH1/2* mutations, but $IDH2^{R140Q}$, are associated with reduced survival in AML patients [55].

Although the hypothesis that *IDH1/2* mutations are directly causing prolonged patient survival is controversial, a causal relationship should be considered. We propose that elucidation of the differences between 1) *IDH1/2*-mutated and *IDH1/2* wild-type cancer biology and 2) the various types of cancer in which *IDH1/2* mutations are associated or not with differences in survival will provide hints regarding whether or not *IDH1/2* mutations are causative in prolonged patient survival and

which mechanisms contribute to it. Increased understanding of the putative survival-prolonging effects of *IDH1/2* mutations may reveal novel therapeutic targets for *IDH1*/2-mutated and *IDH1/2* wild-type cancers. Prolonged survival can have two possible causes: an intrinsic effect (i.e. slower tumor growth due to disturbed cellular metabolism), or an extrinsic effect (i.e. sensitization to therapy). This section discusses both possibilities.

6.1. Intrinsic effects of IDH1/2-mutated tumors

In the case that *IDH1/2* mutations cause a prolonged survival because *IDH1/2*-mutated tumors are less aggressive than their wild-type counterparts, this can be attributed to either 1) slowly proliferating cancer cells or 2) prognostically favorable histological and radiological features. Both options are reviewed.

6.1.1. Slow cellular proliferation

In vitro investigations have not yet unequivocally shown whether or not *IDH1/2* mutations intrinsically inhibit tumor growth. Both a diminished [145–147] and increased proliferation [148,149] after transfection of U87 glioblastoma cells with IDH1^{R132H} have been reported. The relation between *IDH1* mutations and tumor growth was investigated in glioblastoma patients as well. MRIs of treatment-naïve patients with glioma-like symptoms at the time of clinical diagnosis and shortly before surgery showed that *IDH1* mutations are not associated with differences in spontaneous tumor growth [150].

In the case that *IDH1/2* mutations intrinsically inhibit spontaneous tumor growth, the underlying mechanisms are unclear. It may be attributed to suppressed expression of branched-chain amino acid transporter 1 (BCAT1) in *IDH1/2*-mutated glioma [151]. BCAT1 protein expression is mutually exclusive with *IDH1/2* mutations and promotes the catabolism of branched-chain amino acids, which is associated with increased proliferation in other types of cancer, such as mamma carcinoma [152–154]. Alternatively, the impairment of *IDH1*-mutated cancer cells to generate citrate from glutamine [40] may hinder lipid formation and thus cellular proliferation in certain (i.e. hypoxic) contexts.

6.1.2. Favorable histological and radiological features

When *IDH1* mutations were discovered in glioblastoma, it was immediately recognized that they occurred in younger patients, as compared with *IDH1* wild-type glioblastoma (33 versus 63 years) [1]. Although age is an established favorable prognostic factor in glioblastoma [155], *IDH1* mutations are independent prognostic factors in multivariate analyses that control for age [35,156,157].

Histological comparison of *IDH1* wild-type and mutated primary glioblastoma showed that *IDH1*-mutated glioblastoma contain less necrosis and vascular abnormalities occur less frequently. Preoperative MRIs revealed that *IDH1*-mutated glioblastoma are larger and show less edema and necrosis, and more frequently a non-enhancing tumor component, indicating less blood–brain barrier destruction [158]. The last three features are known to be prognostically favorable and one study even suggested that *IDH1* mutations are not independent prognostic factors when controlled for the extent of edema [159]. These radiological features can be explained by the molecular effects of *IDH1/2* mutations. Edema and tumor enhancement are induced by high vascular permeability, which is regulated by vascular endothelial growth factor (VEGF). VEGF expression is dependent on HIF1α, which is degraded in *IDH1/2*-mutated cells [18]. Indeed, VEGF levels are lower in secondary glioblastoma compared with primary glioblastoma [110]. *IDH1/2*-mutated glioma may have less necrosis because the lower HIF1α levels suppress hypoxia-mediated responses to coagulation, a cascade that ultimately leads to necrosis [160]. The larger preoperative tumor size suggests that *IDH1/2*-mutated glioblastoma can grow larger until they become clinically manifest. Most glioblastomas are diagnosed when patients experience neurological symptoms. The lesser extent of edema may explain why *IDH1/2*-mutated glioma can grow larger without provoking symptoms.

Patients with *IDH1*-mutated glioma have been reported to have an improved survival after maximal safe tumor resection, as compared with wild-type *IDH1* glioma patients [161]. In contrast, other studies described that the association between *IDH1/2* mutations and prolonged glioma patient survival is independent of the extent of resection [35,44,157,162]. Overall, the literature on effects of *IDH1/2* mutations on tumor aggressiveness is inconclusive at this moment.

6.2. Sensitization to therapy

Several studies reported that *IDH1* mutations predict glioma responses to temozolomide (TMZ) [163–166] and/or IR (chemoradiation) [167], whereas other studies reported that *IDH1* is a prognostic factor for prolonged survival independently of therapy [35,157,168,169]. When interpreting contrasts between predictive and prognostic values of *IDH1/2* mutations, it is important to remember that the majority of *IDH1*/2-mutated glioblastoma patients are young and all patients are treated according to the Stupp protocol: TMZ and 60Gy of IR [35,170], but the *IDH1/2*-mutated glioblastoma patient cohorts are usually small. Therefore, it is hard to perform sufficiently powered analyses, and definitive conclusions have not yet been made regarding whether or not *IDH1* mutations are prognostic factors independently of therapy.

Mutant IDH1/2 is associated with a prolonged survival in glioma and ICC, but not in AML patients [1,5,23]. Notably, glioma and ICC are treated with IR and adjuvant chemotherapy, whereas AML is treated with chemotherapy only. IR relies more on ROS for sustained cytotoxic effects than chemotherapy. We speculate that therapy sensitization by *IDH1/2* mutations is not observed in AML because AML treatment is less dependent on ROS intermediates for efficacy. This suggests a driver role for ROS effects of *IDH1/2* mutations in treatment response.

6.2.1. Increased sensitivity to alkylating drugs

O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme that reverses the effects of alkylating agents such as TMZ. Methylation of the *MGMT* promoter lowers MGMT protein expression. This phenomenon occurs in ±45% of glioblastoma patients and sensitizes glioblastoma cells to TMZ, resulting in a marked increase in survival of glioblastoma patients treated with TMZ and IR [171].

As described in Section 5.1.2, *IDH1/2* mutations result in global hypermethylation, causing G-CIMP [22,128], which is, in turn, strongly correlated with *MGMT* methylation [127] in both low-grade glioma [128] and glioblastoma [172]. Also, *MGMT* promoter methylation is strongly associated with *IDH1* mutations [163,166,168,173]. A possible explanation for improved survival of *IDH1/2*-mutant glioblastoma patients is that mutant IDH1/2 promotes methylation of *MGMT* by the epigenetic effects of *D*-2HG, thereby sensitizing these patients to TMZ. However, the predictive value of *IDH1* mutations for TMZ treatment is independent of *MGMT* methylation [157,163–166] and *IDH1* mutations are prognostic factors for prolonged survival, independent of *MGMT* methylation status [156,159,165,166,168,172,173]. Furthermore, *MGMT* methylation is not a prognostic factor in WHO grade II and III glioma, whereas *IDH1/2* mutations are associated with improved survival in WHO grade II and III glioma [2]. Finally, *MGMT* promoter methylation was found to be predictive of TMZ response only in *IDH1* wild-type glioma [174].

6.2.2. Increased sensitivity for oxidative stress

Several studies suggest that *IDH1/2* mutations render tumor cells more sensitive for oxidative stress, as induced by 'classical' chemotherapy and IR. In vitro overexpression of IDH1^{R132H} and IDH2^{R172K}, but not wild-type IDH1/2, promotes cell death upon IR [146] or treatment with carmustine (BCNU) [175] or TMZ [176]. This sensitization for therapy was accompanied by elevated intracellular ROS levels and was abrogated by the GSH surrogate N-acetyl-cysteine [175]. Also, patients with

IDH1/2-mutated oligodendroglioma have shown to respond better to procarbazine, lomustine and vincristine [177].

These results are in concordance with studies that report that loss of (wild-type) IDH1/2 function increases cell vulnerability to oxidative stress. Knock-down of IDH1 or IDH2 expression sensitizes HepG2 cells to ethanol-induced cytotoxic stress, which is primarily caused by ROS [178,179]. Furthermore, silencing of *IDH2* in vitro renders HeLa cells more vulnerable to IR and stautosporine [180–182] and sensitizes melanocytes to oxidative stress [183]. Moreover, IDH2 regulates IR-induced apoptosis in murine kidney cells [184]. Conversely, IDH1 overexpression in U87 glioma cells seems protective against TMZ treatment [176]. Additional studies elaborate on IDH1/2 as key enzymes in redox maintenance: *IDH1* and *IDH2* mutations reduce GSH levels in glioma cells [175,185]. Furthermore, in breast cancer stem cells, *IDH1* is upregulated, ROS levels are lower, and radioresistance is increased, as compared with their progeny [186].

Mutant IDH1/2 generates *D*-2HG and consumes NADPH and αKG, two potent antioxidants. In addition, *D*-2HG can induce oxidative stress by itself [137–139]. IDH1 and IDH2 are the most important NADPH providers in glioma cells and most human tissues (but not AML cells; Fig. 3), with a capacity that is two-thirds of the total NAPDH production capacity in glioblastoma. *IDH1/2* mutations decrease the net total NADPH production capacity by 40% [34,35], but do not lower cellular NADPH levels in steady-state conditions [82]. This implies that in steady-state conditions, compensatory mechanisms are sufficient to mend the initial 40% decrease of NADPH production capacity [82]. We speculate that in *IDH1/2*-mutated cells in periods of extreme stress, such as during cancer treatment and especially IR, NADPH demand exceeds NADPH production. As a result, the detoxifying capacity of the cancer cell is insufficient when demand is at its peak, e.g. after acute doses of IR (Fig. 4). This leads to cancer cell death and prolonged patient survival. Mutant IDH1/2 may thus prolong survival by sensitizing cancer cells to treatment by decreasing NADPH production capacity [187] and by lowering the amount of IR-caused oxidative stress which is needed to induce cell death. In contrast to most solid tumors, AML treatment does generally not include IR. Furthermore, G6PDH and not IDH1/2 is the major NADPH generator in AML cells (Fig. 3). This suggests that *IDH1/2* mutations do not greatly affect the reducing power in AML cells and may explain why *IDH1/2* mutations do not prolong survival in AML.

7. Implications of *IDH1/2* mutations for clinical practice

The discovery of *IDH1/2* mutations has resulted in various promising near-future therapeutic options. First, it may be possible to inhibit mutant IDH1/2 [24,25]. Second, better understanding of the effects of *IDH1/2* mutations may unveil novel therapeutic opportunities; it may be possible to enhance the survival-prolonging effects of *IDH1/2* mutations or to mimic such effects in wild-type *IDH1/2* tumors.

7.1. Therapeutic options for IDH1/2-mutated tumors

7.1.1. Mutant IDH1/2 inhibitors

It was recently shown that it may be therapeutically advantageous to inhibit mutant IDH1 or IDH2 in *IDH1/2*-mutated glioma and AML [24,25,115]. The inhibitors only affect activity of mutant IDH1/2 enzymes and leave (healthy) IDH1/2 wild-type enzymes unaffected. This results in slower tumor growth and induces differentiation of cancer cells in vitro and in mouse models [24,25]. Inhibition of mutant IDH1 also enhances apoptosis and inhibits colony formation of *IDH1*-mutated murine cells and primary AML cells [115]. However, the specific conditions in which these inhibitors mitigate differentiation and growth inhibition remain to be elucidated. For example, an IDH1-mutant inhibitor reduced the clonogenic capacity of TS603 glioma cells in one study [24] but had no effect in another [121].

The first clinical trials of IDH2-mutant inhibitors have already started [188]. When these drugs are declared to be safe, *IDH1/2*-mutated cancer patients may be treated with IDH1/2 mutant-specific inhibitors adjuvant to standard treatments. As *IDH1/2* mutations prolong survival in glioma, the possibility exists that mutant-IDH1/2 inhibitors counteract the survival-prolonging effects of mutant IDH1/2. In fact, administration of IDH1/2 mutant-specific inhibitors may even result in an unfavorable clinical outcome, as compared with current treatment. These potential limitations apply to all therapeutic strategies that pursue inhibition of mutant IDH1/2 activity. It needs to be investigated whether mutant-IDH1/2 inhibitors can be used safely as adjuvants. We envision treatments that switch back-and-forth between disjoint periods in which conventional drugs are used subsequently, but not concomitantly, with mutant-IDH1/2 inhibitors. The latter can be used when patients recover from the effects of conventional drugs.

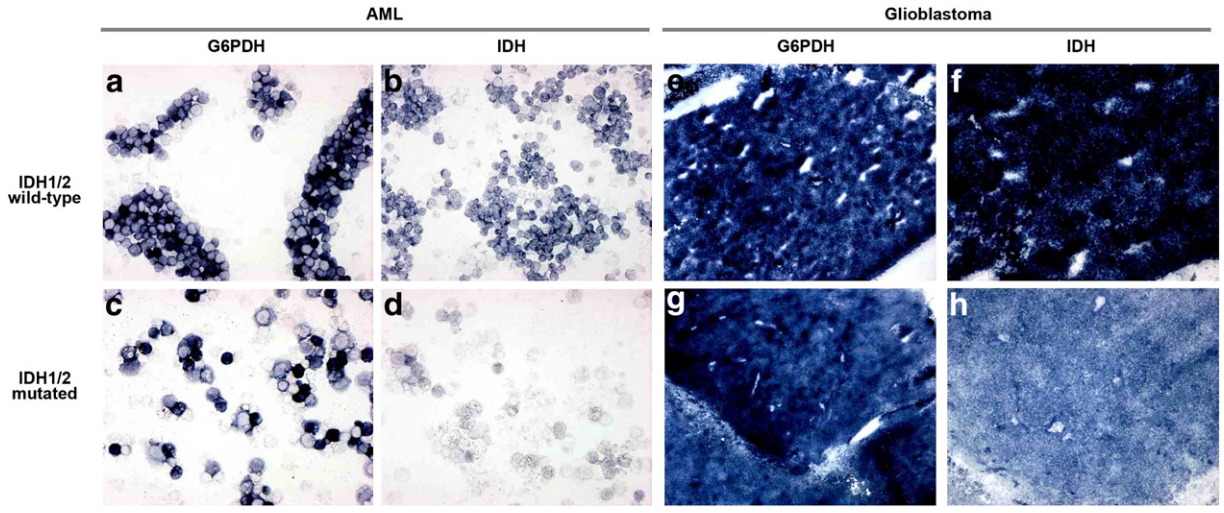

Fig. 3. IDH1 is a more important NADPH producer in glioblastoma than in AML compared with G6PDH. G6PDH (a, c, e, g) and $NADP^+$-dependent IDH1 activity (b, d, f, h) staining of $IDH1^{R132}$ non-mutated (a, b, e, f) and mutated (c, d, g, h) AML cytospins (a–d) and glioblastoma (e–h) cryostat sections. The amount of blue color (nitro BT-formazan) directly reflects $NADP^+$-dependent IDH1/2 and G6PDH activity (production of NADPH).

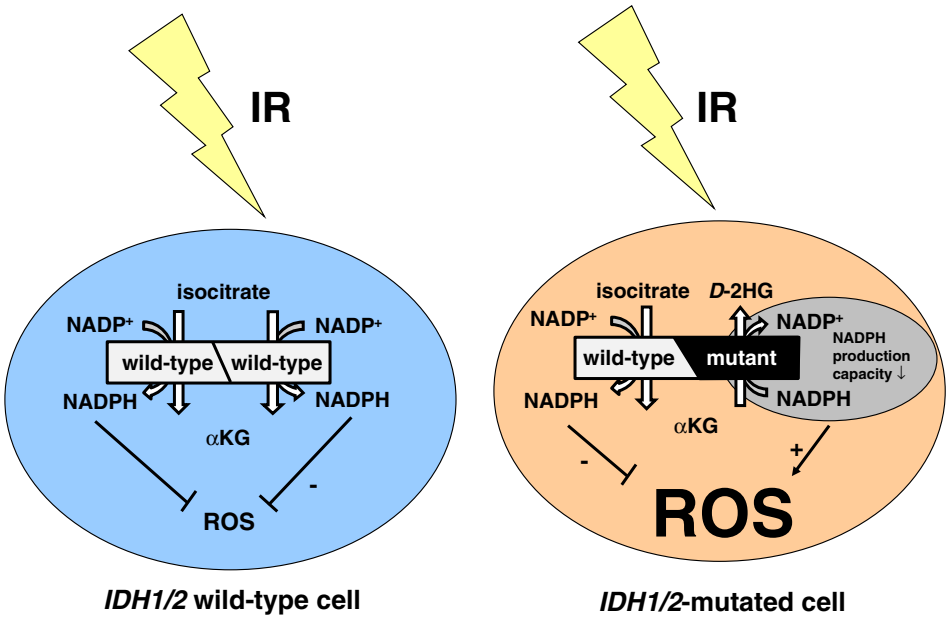

Fig. 4. NADPH generation and consumption in wild-type and mutant IDH1/2 tumor cells. In most human cells, such as glioma cells, IDH1/2 is the most important NADPH producer. NADPH is consumed for detoxification, biosynthetic purposes and *D*-2HG production by IDH1/2 mutants. Although *IDH* mutations do not result in lower cellular NADPH levels under physiological conditions [82], they do cause ~50% decrease in total NADPH production capacity [35] that may become manifest during cancer therapy in *IDH1/2*-mutated glioma cells (orange), resulting in higher levels of ROS in treated *IDH1/2*-mutated cells compared with *IDH1/2* wild-type cells (blue). For patients irradiated during therapy, this may translate to higher *IDH1/2*-mutated tumor cell death and thus prolonged patient survival.

7.1.2. Inhibition of IDH "mutabolism"

IDH1/2-mutated cells rewire their metabolism, either to address the needs of the new *IDH1/2*-mutated phenotype or simply because the loss-of-function of *IDH1/2* mutations reshapes the cellular isocitrate and αKG metabolism. For example, *IDH1/2*-mutated cells need αKG to produce *D*-2HG and are described to be addicted to glutaminolysis as a source of αKG [144,175,189]. Indeed, the glutaminase inhibitor BPTES selectively inhibits growth of IDH1-mutant glioma and AML cells, either by limiting substrate availability to mutant IDH1 or by limiting energy supply in these cells [189,190]. In addition, zaprinast, a PDE5 inhibitor, also targets glutaminase and decreased 2HG levels in *IDH1*-mutated cells [191]. 6-Diazo-5-oxo-L-norleucine (DON) is another inhibitor of glutaminase that is already investigated as chemotherapeutic drug in other types of cancer [192].

It was recently described that *IDH2*-mutated AMLs in mice are susceptible to inhibition of bromodomain-containing protein (Brd4) [88]. BRD4 is an epigenetic regulator of MYC expression, which not only controls cellular proliferation, apoptosis and differentiation at the transcriptional level, but also facilitates glutaminolysis [193]. This suggests that BRD4 inhibition may block glutaminolysis and thus be a therapeutic target in *IDH1/2*-mutated AMLs.

IDH1 mutants increase their TCA cycle flow and are increasingly dependent on oxidative mitochondrial metabolism, as compared to *IDH1* wild-type cancer and non-cancerous cells [40,144]. This renders *IDH1*-mutated cells sensitive to ETC inhibitors, such as rotenone and phenformin. The antidiabetic drug metformin is also a widely-used, cheap and safe ETC complex I inhibitor [194] that could be used to treat *IDH1*-mutated patients.

7.1.3. Demethylating agents

Since it is unknown whether or not the epigenetic effects of *IDH1/2* mutations drive oncogenesis, it remains to be determined whether reversing these epigenetic changes is a therapeutic option. Demethylating agents such as 5-azacytidine and decitabine inhibit proliferation of *IDH1/2*-mutated glioma in vitro and in mice, albeit with high toxicity [121,122]. Two retrospective studies did not find an association between the mutational status of *IDH1/2* and the response to demethylation therapy in 406 and 92 AML/MDS patients [195,196].

7.1.4. IDH1/2 loss of function as a vulnerability

The loss of function of *IDH1/2* mutations may impose a potential vulnerability of glioma cells to further reduction of their NADPH production capacity. This concept has been recently demonstrated in the context of copy number alterations in cancer [197,198], but may also be applicable to *IDH1/2* mutations. Thus, administration of NADPH production inhibitors at doses that would normally be subtherapeutic may selectively sensitize *IDH1/2*-mutated cancer cells for therapy, with only limited side effects. Various compounds that interfere with NADPH production have already been tested in humans. EGCG, a pan-NADPH production inhibitor, can be found in many food supplements and its effects in humans are virtually without noteworthy side effects [199]. Oxalomalate inhibits wild-type IDH1/2 at concentrations that may be unattainable in patients (millimolars) [200] but can serve as a proof-of-concept tool in vitro.

7.1.5. Increasing oxidative stress in IDH1/2-mutated AML

As compared to the current therapy that does not provoke prolonged survival of *IDH1/2*-mutated versus *IDH1/2* wild-type AML patients, it may be beneficial to treat *IDH1*/2-mutated AML more aggressively because these tumors may respond better to heightened levels of stress. For example, targeting cancerous niches of the bone marrow with IR and using total body irradiation in *IDH1/2*-mutated AML may prepare patients better for hematopoietic stem cell transplantations.

7.1.6. Administration of L-2HG (to counteract oncogenic effects of D-2HG)

Another therapeutic strategy may be the administration of *L*-2HG to *IDH1/2*-mutated cancers. It has been reported that *L*-2HG is able to counteract the effects of *D*-2HG on leukemogenesis by inhibiting EGLN [92]. This suggests that administration of *L*-2HG may be a therapeutic option in *IDH1*-mutated AML. However, this approach may be of limited use as *L*-2HG potentially increases the long-term risk of brain tumors in *L*-2HG aciduria [201].

7.2. Therapeutic options for IDH1/2 wild-type tumors

The therapeutic possibilities for *IDH1/2*-mutated cancers are exciting. However, understanding the cancer biology of *IDH1/2* mutations may present therapeutic options for an even larger group of patients: those that have *IDH1/2* wild-type tumors. If we unravel how *IDH1/2* mutations result in prolonged survival in glioma and ICC patients, it may be possible to partially mimic these effects in patients with wild-type *IDH1/2* cancers. Administration of *D*-2HG or inhibitors of NADPH production may achieve such mimicry. Recent advances in kinetics of therapy, such as proton irradiation and hyperthermia-directed release of drugs from nanoparticles could orchestrate these approaches in ways that minimize toxicity [202].

8. Conclusion

8.1. Driver and passenger effects in oncogenesis

There are several possible mechanisms through which *IDH1/2* mutations can promote oncogenesis. Inhibition of αKG-dependent enzyme activity by *D*-2HG seems to be the cornerstone of the oncogenic effects of *IDH1/2* mutations. At this moment, we the most probable driver through which *D*-2HG exerts its oncogenic effects. *D*-2HG promotes EGLN activity and results in HIF1α degradation, which is a tumor suppressor in leukemia and glioma [18,92]. It has been suggested that inhibition of TET2 and JHDMs drives oncogenesis [16,19,22], although recent studies have challenged this hypothesis [24,92]. More research is needed to test whether ECM perturbations and/or increased ROS levels are driving oncogenesis.

8.2. Driver and passenger events in prolonged survival

Statements that *IDH1/2* mutations directly cause prolonged survival of *IDH1/2*-mutated versus *IDH1/2* wild-type cancer patients should be made with caution and are controversial. However, several convincing studies imply *IDH1/2* mutations in increased survival, potentially due to an increased sensitivity for therapy [146,175,178–185] and in favorable histological and/or radiological features, such as low levels of necrosis and edema [158,159]. We speculate that *IDH1/2* mutations may cause a prolonged survival of glioma and ICC, with an increased therapy sensitivity as the most likely driver of these survival-prolonging effects.

8.3. Implications for therapy

Advances in the understanding of the molecular effects of *IDH1/2* mutations have presented several therapeutic options for patients with *IDH1/2*-mutated tumors. IDH1/2-mutant inhibitors abrogate mutant IDH1/2 cancer growth in vitro and in mouse models [24,25]. Patients should be monitored carefully in near-future clinical trials, because IDH1/2 mutant-specific inhibitors may counteract the putative survival-prolonging effects of *IDH1/2* mutations and result in a decrease, rather than an increase in survival. We speculate that such inhibitors may only be used during rest periods between or after conventional ROS-inducing treatments such as IR.

IDH1/2 mutations may also induce other vulnerabilities to tumor cells by generating oxidative and metabolic stress. Potentially, aggravation of this stress, e.g. inhibition of glutaminolysis (BPTES, DON, zaprinast), oxidative mitochondrial metabolism (metformin, phenformin, rotenone) and NADPH production capacity (EGCG) may represent Achilles' heels of *IDH1/2*-mutated tumors.

Ultimately, *IDH1/2* mutations could even present novel therapeutic options for *IDH1/2* wild-type cancers. Of course, such approaches largely depend on the mechanism by which *IDH1/2* mutations prolong survival. For example, inhibitors of NADPH production may mimic the survival-prolonging molecular effects of *IDH1/2* mutations.

8.4. Final remarks

Research on *IDH1*/2 mutations has rapidly evolved from retrospective descriptive investigations reporting *IDH1/2* mutation frequencies and outcomes, through experimental studies on molecular and cellular effects, to IDH1/2-mutant inhibitors that are currently used in clinical trials. We now need to unravel the oncogenic and "survivalogenic" drivers of mutant IDH1/2 cancers and find ways to block the former while preserving or even enhancing the latter. Ultimately, *IDH1/2* mutations may also open doors towards novel therapies in *IDH1/2* wild-type cancers.

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