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*From initial therapy to follow-up*

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Chapter 2: The driver and passenger effects of IDH1 and IDH2 mutations in oncogenesis, metabolic rewiring and survival prolongation

Based on:

Introduction

Over the last decade, genome-wide sequencing of cancers has uncovered causative molecular alterations such as mutations in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) in various types of cancer and genetic (metabolic) diseases. These mutations attracted considerable interest because of research in metabolic reprogramming in cancer and the potential consequences of the neo-enzymatic conversion of α-ketoglutarate (αKG) to D-2-hydroxyglutarate (D2HG) for cancer cells (Figure 1). The accumulating D2HG may competitively inhibit over 60 known αKG-dependent enzymes, causing cellular alterations in epigenetics, collagen maturation, and hypoxia signalling.

This literature review discusses our current understanding of IDH1/2 mutations and their roles in oncosgenesis and prolonged survival. IDH1/2 mutations result in inhibition of a plethora of cellular processes, but many of these may not be relevant for oncosgenesis or prolonging patient survival. In addition, lack of one wild-type IDH1/2 implies less NADPH production, the net result having less capacity to protect cells against oxidative stress. Here, downstream effects of IDH1/2-mutations are evaluated based on whether they are the drivers of cancer initiation or mere passenger effects.

Normal function of IDH1 and IDH2

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 (Figure 1 and 2). 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) and thioredoxins, the formation of active catalase tetramers and activity of cytochrome P450, which are all involved in the reduction of reactive oxygen species (ROS). αKG also possesses detoxifying properties: it reduces H2O2 to water as it decarboxylates to succinate. In most human tissues, particularly the brain, IDH1 is the most important NADPH producer, in contrast to the situation in myeloid cells, where glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) in the pentose phosphate pathway are the major NADPH providers. 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; Figure 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.

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 oncosogenesis have not yet been described.

IDH1/2 mutations in cancer

Glioma. Somatic mutations in IDH1 and IDH2 occur frequently (50-80%) in adult glioma (WHO grade II and III) and secondary glioblastoma (WHO grade IV; Table 1). 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 tumours. Patients with IDH1/2-mutated glioma are on average younger (median age at diagnosis: ~30 years versus ~60 years) and have a relatively favourable prognosis over their counterparts with wild-type IDH1/2 glioma (median survival: 31 months versus 12 months in glioblastoma). In a whole-exome sequencing effort of low-grade glioma and their recurrences, IDH1 mutations were the only persisting mutations in all samples of all patients, indicating that IDH1 mutations are initiating events in low-grade gliomagenesis and as such promising therapeutic targets.

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 90% of the IDH1 mutations are IDH1R132H (Table 1), whereas R132C, R132G, R132L and R132S occur in lower frequencies. In addition, rare IDH1R100Q mutations have been described in four WHO grade II and III glioma, also affecting the catalytic site. The equivalent mutations R172K, R172M and R172W affect the catalytic site of IDH2 on a similar way as the IDH1 mutations.

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**Figure 1. Molecular and cellular effects of mutant IDH1 in a cell and its components.**

Wild-type IDH1 and IDH2 (blue) catalyse the reversible conversion of isocitrate to αKG, with the concomitant production of NADPH from NADP⁺. Mutant IDH1 and IDH2 (orange) convert α-ketoglutarate to D2HG while reducing NADPH to NADP⁺, leading to a decreased NADPH production capacity. D2HG 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.
Figure 2. Chemical effects of wild-type and mutant IDH1/2.

Wild-type IDH1/2 enzymes (arrows in blue) catalyse a two-step reaction in which isocitrate is first oxidised by NADP⁺ to form the intermediate oxalosuccinate and NADPH. Subsequently, IDH1/2 decarboxylates oxalosuccinate to αKG and CO₂. Mutant IDH1/2 enzymes (arrows in orange) possess a neomorphic function: and catalyse a reaction in which αKG is reduced to D2HG. Here, NADPH acts as a hydrogen donor and is oxidised to NADP⁺.

Myeloid haematological 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). In contrast with glioma, IDH2 mutations occur more frequently (~60%) than IDH1 mutations (~40%) in AML, with IDH2R140Q as the most common mutation. In AML, IDH1 mutations correlate with worse patient survival, whereas IDH2R140Q mutations are associated with a moderately prolonged survival. In addition, IDH1/2 mutations are associated with an unfavourable 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, suggesting that IDH1/2 mutations promote malignant transformation to full-blown AML.

Other types of cancer and syndromes. IDH1/2 mutations occur in varying proportions in other types of cancers (Table 1): angioimmunoblastic T-cell lymphoma, acute lymphocytic leukaemia, chondrosarcoma, intrahepatic cholangiocarcinoma, rare cases of colon cancer, gastric cancer, medulloblastoma, non-small cell lung cancer, paraganglioma, and prostate cancer. In addition, IDH1/2 mutations are causative in Ollier disease, Maffucci syndrome and D-2-hydroxyglutaric aciduria (D2HGA). In Ollier disease and Maffucci syndrome patients develop multiple cartilaginous tumours (enchondroma) during childhood. Of note, the occurrence of glioma has been described in both syndromes as well. Haemangioma have been described only in Maffucci syndrome. 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 tumours are derived from these IDH1/2-mutated cells.

D2HGA is an inherited metabolic disease that is caused by heterozygous IDH2R140Q mutations in 50% of the cases and biallelic D2HG dehydrogenase (D2HGDH) mutations in the rest of the cases. D2HGA is characterised by developmental delay, hypotonia, seizures and cardiomyopathy with an onset in the first year of life. Patients often decease in the first decade. The cardiac and neurodegenerative symptoms are directly caused by D2HG accumulation, although the exact mechanisms remain unclear. Another metabolic inherited disease is L-2-hydroxyglutaric aciduria (L2HGA), in which L2HG accumulates, caused by biallelic mutations in the L2HG dehydrogenase (L2HGDH) gene. L2HGA is a clinically milder disease than D2HGA. Of note, L2HGA patients have an increased incidence of glioma, but not of other tumours. A predisposition for gliomagenesis has not been described for D2HGA, but since D2HGA is lethal at younger ages than L2HGA, D2HGA patients may not live long enough to develop glioma.
Table 1. Mutation frequencies of IDH1/2 in different types of cancer.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Preferred subtype for IDH1/2 mutations</th>
<th>IDH1/2 mutated (%)</th>
<th>Mutant alleles</th>
<th>Frequency (%) of total IDH1/2 mutations</th>
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<tr>
<td>Acute lymphatic leukaemia</td>
<td>Not described</td>
<td>5-10</td>
<td>IDH1 R132H</td>
<td>30</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>IDH1 R132G/S</td>
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<td></td>
<td></td>
<td></td>
<td>IDH2, NOS</td>
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<td>IDH2 R172S/W</td>
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<td>IDH2 R172S</td>
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Neomorphic function of mutant IDH1/2

**Mutant IDH1/2 produces D2HG.** Heterozygous IDH1 mutations in glioblastoma result in loss of one wild-type IDH1 allele which reduces the IDH1-facilitated NADPH production capacity by 50%.43,76 Moreover, mutant IDH1/2 causes loss of function through dominant-negative inhibition of wild-type IDH1/2.17,77 In addition, IDH1/2 mutations confer a neomorphic 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.77 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 the fact that αKG is reduced but not carboxylated. Thus, the reaction yields 5-carbon D2HG rather than 6-carbon isocitrate and oxidises NADPH to NADP+.13 All IDH1/2 mutations shown above have this gain of function13,78,79 and exclusively produce the D- (or R-) enantiomeric isof orm of 2HG, and not the L- (or S-) enantiomer (Figure 2).13 Until now, there have been no reports of L2HG-producing IDH1/2 mutations, either naturally occurring or constructed in the laboratory.

Accumulation of D2HG is already being utilised diagnostically in oncology clinics. D2HG in serum and urine correlates with AML and intrahepatic cholangiocarcinoma disease recurrence and can be used to determine the IDH1/2 mutation status.79-83 Accordingly, proton or 13C magnetic resonance microscopy can detect intracranial D2HG accumulation in IDH1/2-mutated glioma.84,86
**IDH1/2 mutants differ in D2HG production.** Mutated IDH1/2 (IDH1_R132, IDH2_R140, IDH2_R172 and their variants) have varying enzymatic properties in terms of D2HG production efficiency. The most common IDH1/2 mutants in glioma and AML (IDH1_R132H and IDH2_R140Q, respectively) are weak D2HG producers, as compared to other variants. For example, the most common IDH1/2 mutant in chondrosarcoma (IDH1_R132C), produces relatively high levels of D2HG. The only IDH1/2 mutant that is found in the germline inherited metabolic disease D2HG aciduria is the weakest D2HG-producing IDH2_R140Q mutant. It has been proposed that only low D2HG levels as a result of such mutations are compatible with embryogenesis; IDH1/2 germline mutations leading to high D2HG levels may be embryonically lethal. It has been hypothesised that in oncogenesis, the intracellular D2HG concentration that gives the largest growth advantage varies, depending on the tumour’s original cell type. This may explain why each type of cancer has a favourite IDH1/2 mutation. In addition, the IDH1/2 mutation and the subsequent D2HG levels may determine which specific type of cancer is being formed.

A great variety of IDH1/2 mutants can be conceived, spanning a spectrum of D2HG accumulation levels. Thus, optimal D2HG concentrations alone do not satisfactorily explain why IDH1 mutations are much more prevalent than IDH2 mutations in glioma, intrahepatic cholangiocarcinoma and chondrosarcoma. Another reason may be that in hypoxia, IDH1/2 can operate in reverse to use αKG to produce citrate, which is subsequently used for fatty acid production. αKG does not need to be produced from glucose but can also be derived from glutamine or glutamate in an anaplerotic pathway called glutaminolysis. 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 catalyse reductive carboxylation, whereas A549 lung carcinoma cells, HCT116 colon carcinoma cells, MF10A mammary epithelial cells and MDA-MB-231 mammary carcinoma cells use IDH1 for that purpose. The reason why some cells prefer IDH1 or IDH2 over the other for reductive carboxylation is unknown at this moment.

**Oncogenic effects of IDH1/2 mutations**

Because IDH1/2 mutations are described as inaugural events in gliomagenesis and occur in premalignant myeloid disorders, direct evidence for oncogenic properties of IDH1/2 mutations was presented by retroviral-mediated introduction of IDH2_R140Q or IDH2_R172K in 10T1/2 mesenchymal progenitor cells, which induced the formation of sarcoma-like tumours and AML in mice. IDH2_R140Q has been shown to be necessary for AML maintenance in mice, indicating that IDH1/2 mutations are therapeutic targets in IDH1/2-mutated cancers. Moreover, identification of downstream oncogenic drivers of IDH1/2 mutations may facilitate rational anti-cancer drug design.

**Interference with αKG-dependent dioxygenases.** The consensus mechanism of IDH1/2 mutation-induced oncogenesis is that D2HG may competitively inhibit or activate some 60 αKG-dependent dioxygenases that are involved in many cellular processes. Four types of αKG-dependent dioxygenases and their putative roles in oncogenesis are discussed here: EGLN, TET2, JMJC and collagen hydroxylases. The oncogenic properties of D2HG have also been shown by the finding that
2HG accumulation also occurs in IDH1/2 wild-type breast cancers and correlates with a worse prognosis in these tumours.95

**Induction of HIF1α degradation by EGLN prolyl-4-hydroxylases.** D2HG accumulation promotes degradation of HIFs. This may occur via induction of egg-laying defective nine (EGLN, also called prolyl hydroxylase domain 2 [PHD2]) prolyl-4-hydroxylase activity,31,96 although the mechanism is not completely clear yet.57 HIF transcription factors mediate responses to hypoxia. EGLN hydroxylates proline residues in HIFs that ultimately lead to the proteosomal degradation of HIFs.98 Accordingly, the expression of 87 HIF1α-responsive genes was markedly decreased in IDH1-mutated glioma as compared to IDH1 wild-type glioma.99

An early effect of IDH1R132H is increased HIF1α levels77 but afterwards, HIF1α levels are suppressed at 8-15 passages after IDH1 R132H transfection *in vitro*.31 HIF expression is upregulated in hypoxic conditions and promotes expression of genes that are involved in all hallmarks of cancer,9,100 indicating its oncogenic role. On the other hand, there is mounting evidence that HIF1α is a tumour suppressor in leukaemia.101-103 In line with this, people living at high altitude and thus residing in a state of chronic hypoxia, presumably have higher levels of HIFs and a lower risk of developing leukaemia.96 *IDH1/2* mutations may contribute to leukaemogenesis by destabilisation of HIF1α. In addition, preclinical evidence suggests that HIFs have tumour-suppressive functions in glioma as well.31,104,105 Necrosis is one of the histological hallmarks of glioblastoma.106 Necrosis is the result of hypoxia-mediated activation of the coagulation system, which causes intravascular thrombosis. This further increases intratumoural hypoxia and leads to abnormal proliferation of endothelial cells and tumour necrosis.107 Remarkably, secondary glioblastoma display much lower rates of necrosis than primary glioblastoma,107 but much higher rates of *IDH1/2* mutations.12,17 In accordance with activation of EGLN by D2HG, HIF is markedly decreased in secondary versus primary glioblastoma.108 This may be the result of less hypoxia response-induction of necrosis in secondary glioblastoma than primary glioblastoma because the frequently occurring *IDH1/2* mutations suppress HIF1α levels and hypoxia responses in secondary glioblastoma.

**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 D2HG (reviewed elsewhere33). D2HG inhibits TET2 activity *in vitro*.30,31 TET2 hydroxylates 5-methylcytosine to 5-hydroxymethylcytosine, which mediates DNA demethylation.32 Somatic inactivating mutations in TET2 are common in myeloid haematological disorders, occurring in 50-60% of secondary AML, MDS and MPN.109-113 TET2 mutations and *IDH1/2* mutations are mutually exclusive in AML,32,112 suggesting that these genes have a similar function. When TET2-mediated DNA demethylation is inhibited by D2HG, DNA becomes hypermethylated.30 Indeed, *IDH1/2* mutations are strongly correlated with global DNA hypermethylation in AML, glioma, intrahepatic cholangiocarcinoma and chondrosarcoma. This induces oncogenesis by repression of differentiation genes and induction of the expression of stem cell maintenance genes,5,32 as is shown by a block in cellular differentiation *in vitro* in IDH1-mutated cells.35

**Histone modifications by JMJC histone demethylases.** D2HG also inhibits αKG-dependent jumonjiC (JMJC)-domain containing histone lysine demethylases (JHKDMs).29,30,35 These demethylases are considered to be tumour suppressors and their inactivation has been associated with oncogenesis in various types of cancer.114 JHKDMs modify chromatin to regulate gene expression epigenetically. In non-cancerous cells, tumour-suppressor genes may be part of the transcriptionally active euchromatin and oncogenes may be part of the transcriptionally silent heterochromatin. Inhibition of JHKDMs by D2HG may disturb this balance. Expression of the Jumoji domain-containing lysine histone demethylases KDM2B and KDM3B is decreased in glioblastoma115 and AML,116 as compared with healthy tissue. This suggests that (JH)KDM inhibition may have oncogenic functions in cancer.
Introduction of mutant IDH1/2 or D2HG increases lysine histone methylation and blocks cell differentiation in vitro. However, it is unclear whether inhibition of JHKDMs by D2HG results in epigenetically relevant changes in vivo; expression of some methylation markers was increased in IDH1R132H knock-in mice (H3K79me2, H3K36me3, H3K4me3) whereas expression of others remained unchanged (H3K27me3, H3K9me3). This suggests that the inhibitory effect of D2HG on JHKDMs may be sufficient to induce methylation changes of at least some histones. The observed D2HG levels may not significantly alter histone methylation in cancer in vivo, or specifically target biologically important loci that promote tumour 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.

**ECM disturbances by collagen hydroxylases and matrix metalloproteinases.** D2HG inhibits three types of collagen hydroxylases; the PLOD, Leprechaun and P4HA families of prolyl hydroxylases, which are required for proper collagen protein maturation. Matured collagen is an important component of the extracellular matrix (ECM), which is heavily involved in cellular proliferation and differentiation. Thus, perturbations in ECM homeostasis may induce oncogenesis. In addition, IDH2R172G mutations have been shown to upregulate expression of MMP2 and MMP9, matrix metalloproteinases that degrade the ECM in relation to carcinogenesis and invasion.

It has been proposed that IDH1/2 mutations and their impact on the tumour microenvironment may explain the diffuse nature of glioma. However, a caveat is the absence of collagen in normal brain parenchyma. Furthermore, wild-type IDH1/2 glioblastoma also have a diffuse infiltrating capacity. ECM disturbances may also be hard to reconcile with the formation of haematopoietic cancers, because one would not expect the ECM to be an important factor in the development of leukaemia. In contrast, the collagen gene COL2A1 is frequently mutated in chondrosarcoma, suggesting that ECM disturbances function in the development of such cartilaginous tumours.

**Increased intracellular ROS levels.** In glioblastoma, IDH1 mutations decrease the production capacity of NADPH, a critical component for cellular detoxification processes. Cells use NADPH to keep glutathione in its reduced form (GSH) as well as thioredoxin. GSH is used by peroxidase and peroxiredoxin to reduce ROS. In addition, D2HG also induces oxidative stress directly although the mechanisms are unclear. As described, IDH1/2 mutations induce loss of function through a dominant-negative inhibition of wild-type IDH1/2. This has been attributed to structural changes of wild-type IDH1/2 due to heterodimerisation with mutant IDH1/2. We speculate that D2HG inhibits wild-type IDH1/2 because it competes with isocitrate due to αKG mimicry, similar to the competitive inhibition by D2HG of αKG-dependent dioxygenases such as TET2 and JMJC.

ROS create 8-oxoguanine lesions in DNA that can lead to mutations that promote cellular proliferation, evasion of apoptosis or anoikis, angiogenesis, tissue invasion and metastasis. This has also been observed in neural stem cells. 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 leukaemia, the hallmark Philadelphia chromosome (BCR-ABL) induces ROS formation and transforms the cancer to an AML-like blast phase.

IDH1 mutations induce mitochondrial dysfunction and increase the number of mitochondria, which are the primary source of ROS. Overexpression of mutant IDH1 results in increased ROS levels in haematopoietic cells. In vitro and in vivo studies have shown that IDH1/2 mutations do not alter ROS levels in immortalised human astrocytes or in brains and haematopoietic cells of IDH1R132H knock-in mice, as compared with wild-type mice. However, these studies used a ROS marker (CM-H2DCFDA) that is insensitive to H2O2. On the basis of NADPH consumption by peroxidase and peroxiredoxin reactions, one would expect H2O2 levels to be elevated in cells depleted of NADPH. The
concept of increased ROS as a driver of malignant transformation in IDH1/2-mutated cancers is very interesting, but mechanistic evidence is required for definitive conclusions.

**IDH1/2 mutations and metabolic rewiring of cancer cells**

The role of D2HG in gliomagenesis has received considerable attention, but direct effects of IDH1/2 mutations on cellular metabolism have been less frequently studied. Effects of IDH1/2 mutations on cellular metabolism have mainly been studied using cells in which recombinant mutant IDH1/2 was overexpressed, or in which cell-permeable analogues of D2HG were used in vitro. Studies in which the IDH1/2 mutant was overexpressed have to be interpreted with caution because IDH1/2 mutations in glioma always occur in a heterozygous fashion, and the wild-type enzyme is needed to provide the mutant form with sufficient αKG substrate. Therefore, the wild-type:mutant ratio may be an important factor in studies of metabolic pathways in IDH1/2-mutated cells.

Since mutated IDH1/2 converts αKG to D2HG with concomitant consumption of NADPH, αKG and NADPH levels are expected to be reduced in the cytosol of glioma cells which must have an impact on cellular metabolism. Excessive consumption of αKG is an important determinant in IDH1/2-mutated cancer cells as well. Besides converting isocitrate to αKG, IDH1/2 can also catalyse the reverse reductive carboxylation of αKG to isocitrate, which is subsequently used for the generation of citrate for lipid synthesis. As lipid synthesis is fundamental for proliferating cells and cellular maintenance, reduced citrate production is expected to have a significant impact on cell survival. IDH1R132H lacks the reverse reaction activity and IDH1-mutated cells may therefore depend on mitochondrial IDH2 for citrate production. We have previously shown that our E478 glioma xenograft model, carrying an endogenous IDH1R132H mutation, presents with high densities of mitochondria supporting the concept of mitochondrial biosynthesis as a rescue mechanism to produce αKG and citrate via IDH2. This rescue mechanism is further supported by our observation that, although αKG levels in IDH1/2-mutated xenografts are slightly lower than in wild-type xenografts, they are still higher than in normal brain tissue. Threshold levels of αKG are a prerequisite for cancer cell survival and proliferation, and therefore these αKG levels in IDH1/2-mutated glioma are not a surprise.

**Figure 3. IDH1 is a more important NADPH producer in glioblastoma than in AML compared to G6PDH.**

![Image of staining](image)

NADP+-dependent G6PDH activity (a, c, e, g) and NADP+-dependent IDH1 activity (b, d, f, h) staining of IDH1R132H 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 colour (nitro BT-formazan) directly reflects NADP+-dependent IDH1/2 and G6PDH activity (production of NADPH).
**Glutamine metabolism in IDH1/2-mutated glioma.** The effects of overexpression of mutant IDH1/2 or exogenous D2HG on glioma cell metabolism have only been addressed in a few studies. Reitman et al. showed that overexpression of IDH1R132H in human oligodendroglialoma cells results in increased levels of D2HG and reduced levels of glutamate and N-acetyl-aspartyl glutamate (NAAG), a common dipeptide in brain which is derived from glutamate. In contrast, exogenous D2HG raises cellular glutamate levels, indicating that glutamate alterations are not a direct D2HG effect but are caused by other IDH1/2 mutation-related processes. IDH1/2-mutated cancer cells are more sensitive than IDH1/2 wild-type cells to inhibition of glutaminase, the enzyme that converts glutamine to glutamate. As glutamate can be directly converted to αKG via glutamate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase, a model of glutamine addiction has been proposed, in which glutamine, via glutamate, is used as fuel to maintain sufficient levels of αKG needed for cell proliferation. The conversion of glutamate to αKG may explain the low steady state concentrations of glutamate and NAAG in IDH1/2-mutated cells.

**Glutamate as a source of αKG?** All metabolic studies so far indicate that IDH1R132H causes metabolic stress in glioma cells by depletion of the cytosolic αKG pool. IDH1/2-mutated tumours therefore need compensatory mechanisms for progression, and possibly only those tumours that have adapted become clinically manifest. One of these compensatory mechanisms may be upregulation of glutamine import and glutaminase activity. However, in vitro studies have shown that inhibition of glutaminase has only a modest effect on the proliferative capacity of IDH1/2-mutated glioma cells. Whether IDH1/2-mutated glioma has high glutaminase activity has not been studied very well, but preliminary results are shown in Chapter 5. Nevertheless, the question remains why cancer cells embark on glutamine import for glutamate production when they are actually bathing in glutamate: glutamate is an important neurotransmitter and is present in synaptic clefts but also in white matter, a location where diffuse infiltrative glioma growth often occurs. There is circumstantial evidence that IDH1/2-mutated cancer cells import glutamate from their microenvironment. Low-grade glioma, of which > 80% carry IDH1/2 mutations, express the excitatory amino acid transporter 2 (EAAT2). This transporter is normally expressed by astrocytes to remove excess glutamate from the interstitium to prevent excitotoxicity. Expression of EAAT2 by low-grade glioma cells is at least suggestive of the capacity of these cells to import glutamate from the surroundings. Therefore, we propose that glutamate acts as a chemotactic compound for glioma cells and contributes to diffuse infiltrative growth. When these assumptions are correct, they provide a strategy for therapy. The glutamate to αKG-conversion can be effectively blocked by relatively inexpensive and safe inhibitors of glutamate dehydrogenase such as epigallocatechin gallate (EGCG, an extract from green tea) and chloroquine (an anti-malaria drug). As both compounds have additional anti-tumour properties by increasing oxidative stress and inhibiting autophagy, studies of their putative anti-glioma activity are warranted.

**Oxidative phosphorylation.** Oxidative phosphorylation is the most efficient method to generate ATP from glucose and its activity is regulated by NADH input from the TCA cycle, the generation of a transmitochondrial membrane proton gradient by the electron transport chain (ETC) and the utilisation of this gradient by ATP synthase (complex V of the ETC) to phosphorylate ADP into ATP. The main source of NADH is the TCA cycle, and although IDH1/2 do not generate NADH, they perform the same isocitrate-to-αKG conversion as the traditional TCA cycle enzyme IDH3. Furthermore, IDH1/2 mutations confer a loss of function of wild-type IDH1/2 kinetics and redirect carbon metabolites away from the TCA cycle and oxidative phosphorylation towards D2HG production, as is shown by decreased expression of TCA cycle enzymes downstream of IDH1/2/3 and decreased oxidative metabolism in Seahorse metabolic assays of IDH1/2-mutated cancer cells (Chapter 3). IDH1 mutant-induced mitochondrial dysfunction is also supported by a compensatory increase in the number of mitochondria in IDH1/2-mutated cells. The result is that IDH1/2-mutated cancer cells are vulnerable to inhibition of residual oxidative metabolism with inhibitors of the ETC, such as the biguanides metformin and phenformin that inhibit NADH dehydrogenase (complex I).
**Inhibition of oxidative phosphorylation using metformin.** Metformin has been used for the treatment of type 2 diabetes mellitus (T2DM) since the 1970s. The drug decreases blood glucose levels by suppressing gluconeogenesis in the liver, increasing uptake of circulating glucose in the muscles and increasing insulin sensitivity. Metformin has recently attracted interest as a chemopreventive drug for various types of cancer since retrospective studies have established that patients suffering from T2DM who were treated with metformin had a lower incidence of cholangiocarcinoma, pancreatic cancer, colon cancer and breast cancer in comparison with diabetic patients that did not receive metformin. Another cohort study showed that metformin treatment of T2DM patients led to a significant decrease in overall cancer risk and onset time, and that these effects were dose dependent. The molecular mechanisms of metformin treatment are still poorly understood, but there is considerable evidence that metformin exerts its antineoplastic effects via inhibition of complex I. For example, tumour growth is not inhibited in mouse xenografts of human cancer that chimerically express a biguanide-resistant yeast type of complex I instead of human complex I. Furthermore, proliferation was not inhibited in cancer cells grown under hypoxic conditions or lacking mitochondrial function and cancer cells that were unable to upregulate oxidative phosphorylation under hypoglycemic conditions were more sensitive to metformin treatment, which further suggests that inhibition of oxidative phosphorylation is a key component of the anti-cancer effects of metformin. Moreover, metformin uncoupled respiration in the mitochondrion, which by itself inhibited proliferation of breast cancer cells. The precise mechanism how decreased ETC activity leads to decreased cancer cell proliferation remains subject of debate. Because oxidative phosphorylation is the most efficient method for cells to generate ATP from glucose, decreased cancer cell proliferation after metformin treatment may be the result of energetic stress due to decreased ATP levels, which restricts the amount of energy that cancer cells have at their dispose to proliferate. For example, metformin-treated cells increase their glucose uptake and lactate production, which is indicative for a metabolic shift towards glycolysis, which is less efficient in generating ATP from glucose. It seems plausible that IDH1/2-mutated cancer cells, which are already being metabolically and energetically challenged due to the metabolic rewiring by IDH1/2 mutations, are sensitive to metformin treatment because metformin increases intracellular metabolic stress.

**Conclusion**

There are several possible mechanisms through which IDH1/2 mutations can promote oncogenesis. Inhibition of αKG-dependent enzyme activity by D2HG seems to be the cornerstone of the oncogenic effects of IDH1/2 mutations. At this moment, we consider EGLN as the most probable driver through which D2HG exerts its oncogenic effects. D2HG promotes EGLN activity and results in HIF1α degradation, which is a tumour suppressor in leukaemia and glioma. It has been suggested that inhibition of TET2 and JHDMs drives oncogenesis, although recent studies have challenged this hypothesis. More research is needed to test whether ECM perturbations and/or increased ROS levels are driving oncogenesis. The metabolic rewiring that is the result of direct metabolic effects of IDH1/2 mutations (through abrogation of IDH1/2 wild-type kinetics, D2HG accumulation and αKG depletion) or their epigenetic effects (via inhibition of TET2 and JHDMs) may not directly contribute to IDH1/2 mutation-mediated oncogenesis, but may represent interesting targets for future therapies. Inhibition of ETC activity using oral biguanides such as metformin and inhibition of glutaminolysis using chloroquine may be the most promising example, because these drugs can relatively easily be repurposed for the treatment of IDH1/2-mutated cancers, when proven safe and effective. In summary, 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. Ultimately, knowledge on the effects of IDH1/2 mutations may also open doors towards novel therapies in IDH1/2 wild-type cancers.