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Review

# Glutamate as chemotactic fuel for diffuse glioma cells: Are they glutamate suckers?

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ABSTRACT

Diffuse gliomas comprise a group of primary brain tumors that originate from glial (precursor) cells and present as a variety of malignancy grades which have in common that they grow by diffuse infiltration. This phenotype complicates treatment enormously as it precludes curative surgery and radiotherapy. Furthermore, diffusely infiltrating glioma cells often hide behind a functional blood–brain barrier, hampering delivery of systemically administered therapeutic and diagnostic compounds to the tumor cells. The present review addresses the biological mechanisms that underlie the diffuse infiltrative phenotype, knowledge of which may improve treatment strategies for this disastrous tumor type. The invasive phenotype is specific for glioma: most other brain tumor types, both primary and metastatic, grow as delineated lesions. Differences between the genetic make-up of glioma and that of other tumor types may therefore help to unravel molecular pathways, involved in diffuse infiltrative growth. One such difference concerns mutations in the $NADP^+$-dependent isocitrate dehydrogenase (*IDH1* and *IDH2*) genes, which occur in >80% of cases of low grade glioma and secondary glioblastoma. In this review we present a novel hypothesis which links *IDH1* and *IDH2* mutations to glutamate metabolism, possibly explaining the specific biological behavior of diffuse glioma.



**Contents**



*Abbreviations:* BBB, blood–brain barrier; GBM, glioblastoma; GDH, glutamate dehydrogenase; IDH, isocitrate dehydrogenase; 2-HG, 2-hydroxyglutarate; α-KG, α-ketoglutarate

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## 1. Introduction

### 1.1. Glioma; histopathology

According to WHO guidelines, diffuse glioma can be characterized as grade II–III oligodendroglioma, grade II–IV astrocytomas, or mixed oligoastrocytomas [1]. Grade II gliomas have low rates of cell proliferation and consequently progress slowly, resulting in a relatively favorable outcome (median survival 12–16 years). Prognosis for patients with grade IV glioma (glioblastoma, GBM) is poor, despite aggressive therapies (median survival < 17 months) [2,3]. Most GBMs arise de novo (primary GBM, ~90%) but GBM may also gradually develop from lower grade tumors (secondary GBMs, ~10%) [4]. The difference between primary and secondary GBM may be important since these have mutually exclusive genetic aberrations which may be utilized for therapeutic purposes, as will be discussed below [5].

Both low- and high-grade gliomas characteristically show tumor cells that grow via diffuse infiltration into the neuropil [6]. This results in perineural satellitosis and migration along white matter tracts, which facilitates tumor cells to cross the corpus callosum. Therefore diffuse gliomas frequently involve both hemispheres. Growth via vessel co-option in the perivascular space of Virchow–Robin is also often observed [7].

### 1.2. Glioma: clinical behavior and treatment

In high-grade glioma, clinical symptoms are a result of, among others, local angiogenesis and associated blood vessel hyperpermeability, a major cause of edema and increased intracranial pressure. Furthermore, high-grade glioma cells may secrete the neurotransmitter glutamate. The resulting supraphysiological concentrations may be toxic to neurons, a phenomenon known as excitotoxicity [8]. Neuronal loss not only directly induces clinical symptoms (seizures, personality changes, loss of neuronal function), but may also generate space for tumor expansion [9].

In the last 40 years, little progress has been made in the treatment of glioma when compared to other tumor types. Standard of care for GBMs is surgical resection to a maximally safe extent, followed by radiotherapy and treatment with the alkylating agent Temozolomide [3,10]. However, due to the diffuse character, individual (groups of) tumor cells are routinely found at sites as far as 2 cm from the main tumor mass [11] and these cells escape magnetic resonance (MR)-based detection as well as surgery and radiotherapy. Therefore, tumors generally recur from the surgical margin and all current therapies for diffuse glioma must be considered as palliative only [12]. It is evidently important to decipher the molecular mechanisms underlying the diffuse infiltrative phenotype of these tumors to tackle this problem and improve the outlook of glioma patients.

The proposed necessity of angiogenesis in solid cancers [13] has provided the rationale for developing a number of angiogenesis inhibitors such as the vascular endothelial growth factor (VEGF) inhibitor bevacizumab, the $\alpha_v\beta_3$-integrin antagonist cilengitide [14] or multifactorial receptor tyrosine kinase (RTK) inhibitors targeting angiogenic pathways. Whereas monotherapy with bevacizumab has turned out to be disappointing in most tumor types, including glioma [15,16], combination with chemotherapy has been shown to prolong progression-free and overall survival in patients with advanced colorectal cancer and some other solid cancer types [17]. For brain tumors, several preclinical and clinical studies have now shown that anti-angiogenic treatment may reduce contrast-enhancement by MRI (indicative of normalization of the blood–brain barrier) [18–20] which is however not necessarily accompanied by anti-tumor effects [21–26]. Indeed, the high density of pre-existent vessels in the brain may allow glioma cells to progress in the absence of angiogenesis due to vessel co-option [7,27] and it is likely that these tumor cells are therefore intrinsically resistant to anti-angiogenic therapies [28]. There is now consensus that these therapies do not inhibit diffuse growth of glioma [29] and several groups even proposed that angiogenesis inhibition would induce an even more invasive phenotype [30,31]. In accordance with this, we observed increased vessel co-option of tumor cells in E98 xenografts under angiogenesis inhibition (compare Fig. 1B/C to the control in Fig. 1A, [22]). Two large phase III studies have recently reported that bevacizumab does not prolong overall survival in glioblastoma patients [32,33]. Altogether, it seems unlikely that bevacizumab will have an important role in clinical practice for treatment of glioma patients [26]. Yet, although anti-angiogenic treatment does not result in anti-tumor effects, it may improve quality of life of glioma patients, presumably by relieving edema-related effects [19,34,35]. In this respect the effect of anti-angiogenic treatment resembles that of corticosteroids, which are routinely given to glioma patients to reduce intracranial pressure [36].

Besides vessel co-option, there are several alternative explanations that may explain the failure of anti-angiogenic therapy; 1) The tumor cells may over-express pro-angiogenic factors that are not targeted by

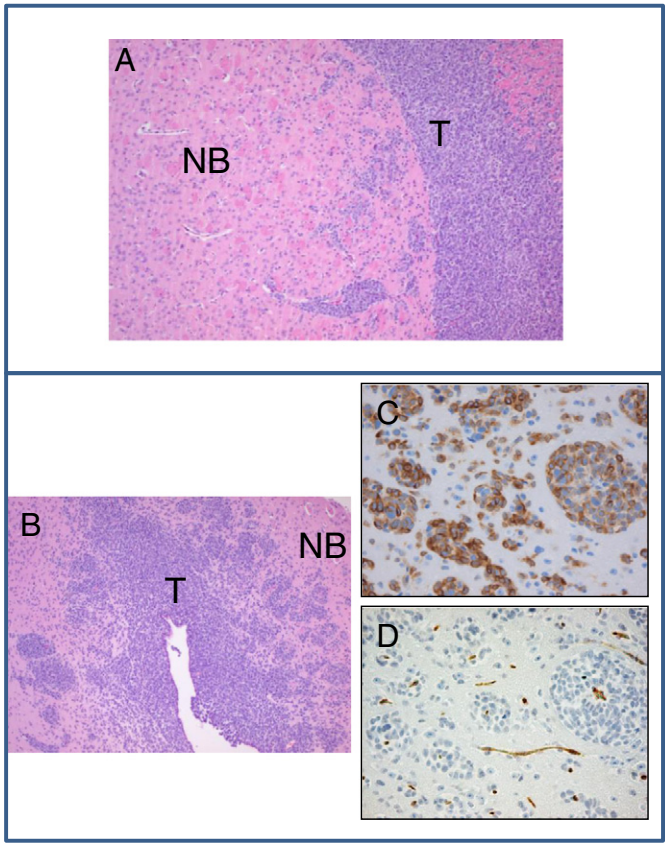


**Fig. 1.** Angiogenesis inhibition increases vessel cooption in orthotopic E98 GBM xenografts. A) H&E staining of the brain of a non-treated mouse showing tumor (T) and normal brain (NB), B–D) H&E, MET and CD34 staining, respectively, of a mouse treated with a VEGF inhibitor. Note that upon treatment a phenotype develops in which tumor cells are arranged as small cuffs (MET-positive; brown staining, C) around blood vessels (CD34 positive, brown staining, D). The immunostainings in C and D represent high magnifications of sections, serial to the H&E staining in B.

therapy; 2) tumors may switch between sprout formation from existing blood vessels and vasculogenesis (involving bone-marrow derived endothelial precursor cells [37]); 3) tumor cells may acquire endothelial cell functions themselves (vascular mimicry [38]); or 4) tumor cells may undergo metabolomic reprogramming towards anaerobic respiration, characterized by increased glycolysis [25,39]. We have previously found in orthotopic glioma xenografts that invasive growth may increase following anti-angiogenic treatment (Fig. 1 and [22]). In combination with vessel normalization (and concomitant restoration of the function of the blood brain barrier) this may actually counteract concomitantly administered therapies [21,27,30]. Anti-angiogenic therapy of glioma is therefore multifaceted. These considerations, again, all point to the necessity of finding novel approaches to inhibit the diffuse infiltrative phenotype of gliomas.

### 1.3. Molecular alterations during glioma development

During the last decade, numerous molecular alterations related to oncogenesis and progression of diffuse gliomas have been discovered and the list is steadily expanding. Mutations in *TP53*, *CDKN2A* and *B* are frequently observed in low-grade glioma and result in dysregulation of apoptosis and promotion of cell survival and proliferation [2,40]. Other aberrations especially frequent in high-grade tumors are mutations and amplifications of the oncogenes *EGFR*, *c-MET*, *c-KIT*, *PDGFRα* and *PTEN* [41–44]. These mutations result in overactivation of downstream signaling pathways, including the RAS–RAF–MAPK pathway (resulting in cell proliferation and migration) and the PI3K pathway (promoting cell survival). Noteworthy is the constitutively-active EGFR variant III (*EGFRvIII*), resulting in a truncated EGFR protein which lacks the protein domains encoded by exons 2–7 and does not require ligand binding for activation [45]. Auto-activating mutations in *PDGFRα* in combination with gene amplification occur relatively frequently in pediatric diffuse intrinsic pontine glioma. In combination with p53 loss, such mutations are sufficient to induce diffuse glioma development in mice [46]. Loss of chromosome arms 1p and 19q (1p/19q co-deletion), often with underlying mutations in both *FUBP1* and *CIC*, is strongly associated with the oligodendroglial phenotype of diffuse gliomas [2,47], yet the biological mechanisms behind this association are still poorly understood.

Over-expression of tyrosine kinase receptors is a tumor-promoting event occurring in a broad range of tumor types [48]. Their exposure on cancer cells has made these receptors attractive therapeutic targets, a prime example being EGFR which is targeted by cetuximab (a humanized antibody that induces receptor internalization and degradation, thereby preventing its activation) [31,49,50]. In glioma, such antibodies may have access to tumor cells and exert therapeutic effects only in areas where the blood–brain barrier is disrupted [21,48]. Whether antibodies can be engineered to acquire sufficient blood–brain barrier penetration capacity to target tumor cells in diffuse areas of glioma remains to be seen. A promising development in this respect is the use of small recombinant llama antibodies [51].

Another class of drugs targeting oncogenic receptors is the small molecule tyrosine kinase inhibitors (TKIs). TKIs with specificity for VEGFR2, EGFR, c-KIT, PDGFRα and c-MET, or combinations thereof, have been developed and are widely applied in oncology practice, some with encouraging results [52]. However, most TKIs that are currently used in clinical practice (e.g. sunitinib, sorafenib, vandetanib, cabozantinib) have VEGFR2 as one of the target receptors and the inhibition of VEGFR2-pathways may cause normalization of the blood–brain barrier that reduces the efficacy of these TKIs in diffuse glioma, as outlined before [22,23,52]. Therefore, specific targeting of gliomas with these TKIs, even of those tumors with high expression levels of targetable oncogenic receptors, may still be of limited value because of concomitant restoration of the blood–brain barrier by these compounds. It stresses the need for the development of glioma-targeting compounds with blood–brain barrier penetration properties. Strategies that allow active or passive transport of therapeutic compounds over the blood brain-barrier (BBB) may include 'smart' development or selection of therapeutic compounds with intrinsic BBB-passage properties, transient disruption of this barrier, inhibition of efflux properties in endothelial cells or convection-enhanced delivery [53].

### 1.4. IDH1 and IDH2 mutations

A breakthrough in our understanding of glioma biology occurred in 2008 when a comprehensive exome sequencing effort revealed heterozygous point mutations in the genes encoding isocitrate dehydrogenase (IDH) 1 or 2 in 70–90% of grades II and III glioma and secondary GBMs [54,55]. The cytosolic IDH1 protein and its mitochondrial counterpart IDH2 are involved in the $NADP^+$-dependent oxidative decarboxylation of isocitrate into α-ketoglutarate (αKG; see Fig. 2). In the human brain, this reaction is estimated to be responsible for the generation of up to 65% of cytosolic NADPH which is needed for biosynthesis of most, if not all, scavengers of reactive oxygen species [56,57]. IDH3 is a different, $NAD^+$-dependent, enzyme that functions in the tricarboxylic acid (TCA) cycle. Thus far, *IDH3* mutations have not been observed in glioma. When we hereafter refer to IDH mutations in this review, this only includes IDH1 and IDH2.

Except for glioma [58], mutations in the *IDH1* or *IDH2* genes are found in a limited number of other tumor types including acute myeloid leukemia (AML, 23%) [59], chondrosarcoma (60%) [60], intrahepatic cholangiocarcinomas and angioimmunoblastic T-cell lymphomas [61]. A potentially important observation is that in gliomas, mutations in *IDH1* are most common (*IDH2* mutations occur at a frequency of only 2–5%) [55] whereas *IDH1* and *IDH2* mutations occur with equal frequency in AML [62]. The large majority of mutations involve an arginine residue at position 132 in the catalytic site of IDH1 which is frequently substituted with histidine (R132H) while *IDH2* mutations mostly involve the equivalent R172 [54,55]. *IDH2* mutations in AML also involve R140, again in the enzymatically active site [59]. These point mutations decrease the affinity of the enzyme for isocitrate, but increase the affinity for αKG [63]. Additionally the mutant enzymes have acquired a novel function by reducing αKG into D-2-hydroxyglutarate (D-2HG) with concomitant NADPH oxidation (Fig. 2 and [64]). Therefore, these *IDH* mutations are predicted to result in:

a) production of D-2HG
b) depletion of αKG
c) decreased NADPH production capacity and increased NADPH consumption.

### 1.5. D-2HG in tumor initiation

Research in the field of IDH mutations in glioma has mostly focused on the consequences of D-2HG production. D-2HG, a byproduct of hydroxylysine catabolism, is rapidly degraded under normal physiological conditions by D-2HG dehydrogenase and cellular levels are normally low [65]. In IDH1-mutated cells, the generation of D-2HG outweighs its removal approximately 1000-fold [64], resulting in its accumulation. αKG is an essential co-factor for a number of enzymes, such as the TET (Ten-Eleven-Translocation) family and Jumonji-C-domain-containing histone demethylases (JHDMs) [66,67]. The structural similarity between αKG and D-2HG in combination with an elevated D-2HG/αKG ratio results in competitive binding of D-2HG and reduced activity of these enzymes in *IDH*-mutated cancer cells (Fig. 2). TET1 and TET2 are hydroxymethylases, converting methylcytosine to hydroxymethylcytosine, a rate-limiting step in demethylation of CpG islands in the genome [68], whereas JHDMs are involved in demethylation of lysines on histone H3. Consequently, D-2HG accumulation in *IDH*-mutated cancer cells results in global DNA and histone hypermethylation, altering gene expression and inhibiting differentiation [69]. These are considered to be the prime oncogenic events in *IDH*-mutated glioma.

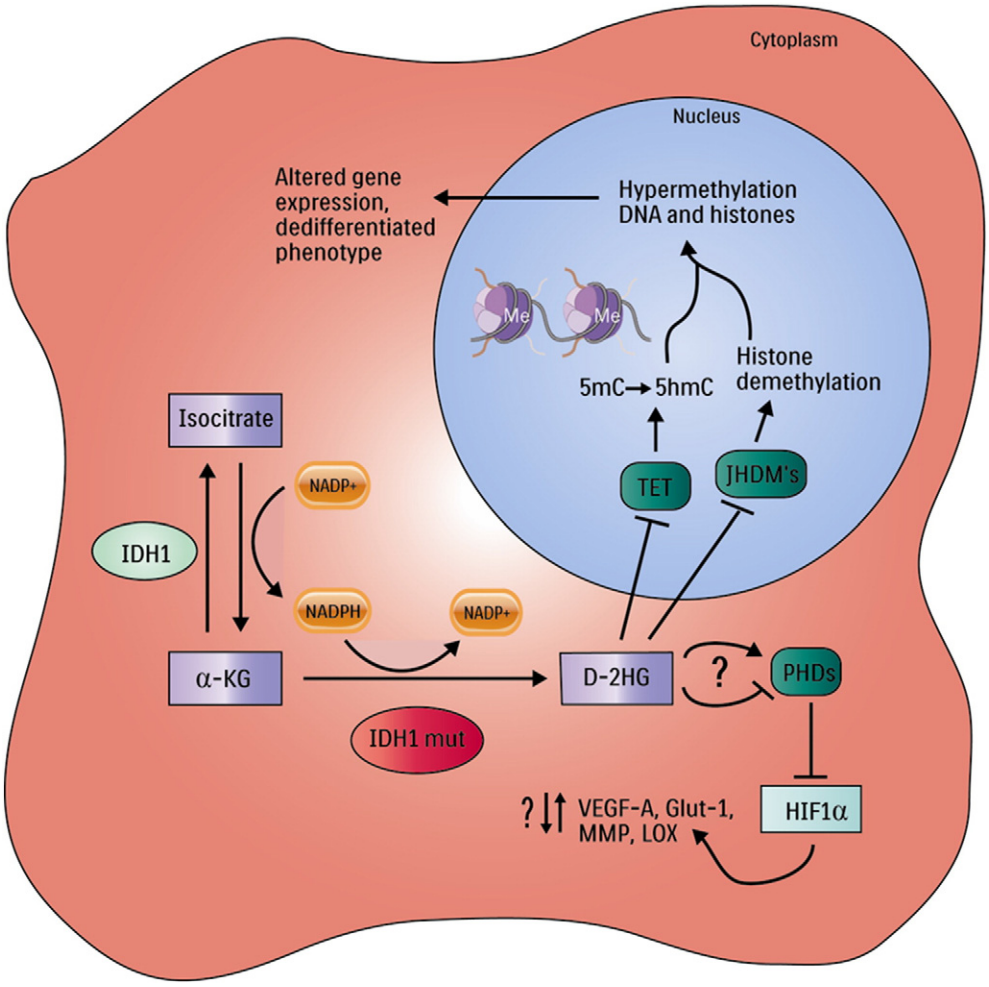


**Fig. 2.** Effects of the oncometabolite D-2HG. IDH1 mutant enzyme has acquired a novel function of reducing αKG to D-2HG. D-2HG binds competitively to enzymes which normally use αKG as a cofactor, thereby reducing activity of these enzymes. Inhibition of TET hydroxymethylases leads to hypermethylation of CpG islands in the genome. Inhibition of JHMDs leads to hypermethylation of lysines in histone H3. This hypermethylated genotype results in altered gene expression and a dedifferentiated phenotype of the cell. Note that both D-2HG-mediated stimulation and inhibition of prolyl hydroxylases (PHDs) has been suggested in literature. As PHDs regulate degradation of HIF1α, this translates into either upregulation or downregulation of hypoxia-inducible genes. The histopathology of IDH1 mutated (low-grade) tumors, which in general lack signs of hypoxia and expression of hypoxia related genes, suggests that D-2HG stimulates, rather than inhibits, PHDs.

In addition, D-2HG serves as a cofactor for EGLN (Egg Laying defective Nine). EGLN, a member of the family of prolyl hydroxylases (PHDs), hydroxylate specific proline residues of hypoxia-inducible factor 1 (HIF1α), causing the protein to be ubiquitinated and degraded [70]. HIF1α is a transcription factor that regulates expression of a number of hypoxia-related genes such as glucose transporter 1 (GLUT-1) and VEGF-A [71] and is a central modulator of angiogenesis and vascular hyperpermeability. Increased HIF1α degradation by D-2HG [70] fits with the notion that *IDH* mutations are frequent in grades II/III glioma that lack necrosis and microvascular proliferation [72,73] (Fig. 2). Furthermore, low-grade *IDH*-mutated gliomas show less edema and contrast enhancement [70,74,75].

As D-2HG results in increased cell proliferation, and exogenous addition of D-2HG results in epigenetic alterations and HIF1α degradation, comparable to the situation in IDH1 mutants, at least in leukemic cells [75], D-2HG is now considered to be an oncometabolite.

### 1.6. D-2HG in tumor progression

Patients with *IDH*-mutated glioma have a better prognosis than patients with *IDH* wild-type tumors [55] and several hypotheses have been postulated to explain this. First, the CpG island methylated phenotype (CIMP) may affect expression of DNA repair proteins such as MGMT (methyl guanine-methyltransferase) [76], thus increasing the susceptibility to DNA-alkylating agents such as Temozolomide, which is standard treatment for glioblastoma. However, the favorable prognosis of *IDH* mutations does not seem to be related solely to Temozolomide responses [77]. Second, a more intriguing explanation is based on the recent finding that, in contrast to *IDH1* wild-type glioma, *IDH1*-mutated glioma do not express branched amino acid transaminase 1 (BCAT1) [5]. BCAT1 is responsible for catabolism of branched amino acids and generates the building blocks needed for cell proliferation and tumor progression, thereby contributing to tumor malignancy. Of note, *IDH* mutations generally do not lead to a survival benefit in AML patients [25,48]. At present, it is not known whether a similar mutually-exclusive relationship between *IDH1* mutations and BCAT1 expression exists in AML.

### 1.7. Metabolic alterations: IDH 'mutabolism'

Whereas the role of D-2HG in gliomagenesis has received considerable attention, direct effects of *IDH* mutations on cellular metabolism have been less frequently studied [65,78]. Numerous attempts, including those in our department, have shown that it is extremely difficult, if not impossible, to generate in vitro cultures of glioma cells carrying endogenous *IDH* mutations, and this has obviously hampered metabolic studies [79–81]. These problems are remarkable since HT1080, a frequently-used fibrosarcoma cell line, carries an $IDH1^{R132C}$ mutation

and produces D-2HG [82]. In addition, several chondrosarcoma cell lines have *IDH1* mutations [83]. This shows that unknown glioma-specific characteristics prohibit in vitro propagation of *IDH*-mutated cells. Contributions by the development of a cre-lox based conditional $IDH1^{R132H}$ knock-in mouse model have been limited since this mutation results in perinatal death [84].

In contrast with the failure to establish IDH1-mutated glioma models in vitro, we have been able to establish a human IDH1-mutated xenograft line from anaplastic oligodendrogliomas that preserves the DNA copy number changes and mutations of the parental tumors. Interestingly, these xenografts grow very slowly in animals, thereby reflecting the tumor behavior in patients. These models may become important tools to study metabolic alterations in an IDH mutator phenotype [47,81].

Effects of *IDH* mutations on cellular metabolism have mainly been studied using cells in which recombinant mutant IDH is overexpressed, or in which cell-permeable analogs of 2HG are used in vitro [75]. Studies in which the IDH mutant is overexpressed have to be interpreted with caution because *IDH* mutations in glioma always occur in a heterozygous fashion, and the wild type enzyme is probably needed to provide the mutant form with sufficient αKG substrate [85]. Therefore, the wild-type/mutant ratio may be an important factor in studies of metabolic pathways in *IDH*-mutated cells.

Since mutated IDH converts αKG to D-2HG with concomitant consumption of NADPH, αKG and NADPH levels are expected to be reduced in the cytosol in glioma cells which must have an impact on cellular metabolism. Cytosolic NADPH, ~65% of which is produced by IDH1 [56], is important for survival as it is the major metabolite needed for detoxification processes that, among others, protect cells from reactive oxygen species (ROS) which are produced especially during radiotherapy and chemotherapy [86]. This may be an additional explanation for the relatively good prognosis of *IDH*-mutated glioma patients. The reduced potential to detoxify ROS would predict that *IDH*-mutated gliomas are more susceptible to radiotherapy than *IDH* wild-type glioma.

Excessive consumption of αKG is an important determinant in *IDH*-mutated cancer cells as well. Besides converting isocitrate to αKG, IDH can also catalyze the reverse reductive carboxylation of αKG to isocitrate, which is subsequently used for the generation of citrate for lipid synthesis [87,88]. As lipid synthesis is fundamental for cell proliferation and maintenance, reduced citrate production is expected to have a significant impact on cell survival. $IDH1^{R132H}$ lacks the reverse activity [64,89] and *IDH1*-mutated cells may therefore for a large part depend on mitochondrial IDH2 for citrate production. We have previously shown that our E478 glioma xenograft model, carrying an endogenous $IDH1^{R132H}$ mutation, presents with high densities of mitochondria supporting the concept of mitochondrial biosynthesis as a rescue mechanism to produce αKG and citrate via IDH2 [81]. This rescue mechanism is further supported by our observation that, although αKG levels in *IDH*-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 tumor cell survival and proliferation, and therefore these αKG-levels in IDH-mutated glioma are not a surprise.

Mitochondrial biosynthesis requires additional production of lipids for membrane synthesis, and results in increased oxidative stress, thus further increasing the demand for αKG and NADPH production. That mitochondrial αKG production is sufficient to maintain levels under conditions in which αKG is continuously converted to D-2HG, is questionable and we therefore hypothesize that additional anaplerotic pathways may be active. Investigations in this direction may open new ways towards novel treatment opportunities.

### 1.8. Glutamine metabolism in IDH-mutated glioma

The effects of overexpression of mutant IDH or exogenous D-2HG on glioma cell metabolism have only been addressed in a few studies. Reitman et al. showed that overexpression of $IDH1^{R132H}$ in human oligodendroglioma cells resulted in increased levels of D-2HG and reduced levels of glutamate and N-acetyl-aspartyl glutamate (NAAG), a common dipeptide in brain which is derived from glutamate [78]. In contrast, exogenous D-2HG raises cellular glutamate levels, indicating that glutamate alterations are not a direct D-2HG effect but are caused by other IDH mutation-related processes. IDH-mutated cancer cells are more sensitive than IDH wild-type cells to inhibition of glutaminase, the enzyme that converts glutamine to glutamate [90]. As glutamate can be directly converted to αKG via glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), 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 [91]. The conversion of glutamate to αKG may explain the low steady state concentrations of glutamate and NAAG in *IDH*-mutated cells [78].

### 1.9. Glutamate as a source of αKG?

All metabolic studies so far indicate that $IDH1^{R132H}$ causes metabolic stress in glioma cells by depletion of the cytosolic αKG pool. *IDH*-mutated tumors therefore need compensatory mechanisms for progression, and possibly only those tumors 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 IDH-mutated glioma cells [90]. Whether *IDH*-mutated glioma has high glutaminase activity is not known and is currently under investigation by us. Nevertheless, the question remains why tumor cells would 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 the white matter [92], a location where diffuse infiltrative glioma growth is often found. There is circumstantial evidence that *IDH*-mutated tumor cells import glutamate from their microenvironment. Low-grade glioma, of which >80% carry *IDH* mutations, express the excitatory amino acid transporter 2 (EAAT2) [9]. This transporter is normally expressed by astrocytes to remove excess glutamate from the interstitium to prevent excitotoxicity [8]. 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.

### 1.10. A comprehensive model for diffuse growth of IDH-mutated glioma cells

Putting all data together, the acquisition of *IDH* mutations in astrocytic or oligodendrocytic (precursor) cells in the brain results in the production of D-2HG which induces the CIMP phenotype and hypermethylated histones, resulting in a block of differentiation. Simultaneous or subsequent mutations in tumor suppressor genes such as p53 and PTEN may then allow true neoplastic transformation. In this scenario, D-2HG is the initiating metabolite, but on the other hand tumor cells need to adapt to the metabolic stress imposed by consumption of αKG and NADPH by mutant IDH. This adaptation may be accomplished by continuous import of glutamate and its conversion to αKG which is subject to a continuous and futile stream of conversion to D-2HG (Fig. 3). Interestingly, the EAAT2 glutamate importer is expressed in low-grade glioma cells in vivo, but expression is often lost in vitro [9]. A lack of glutamate import in vitro may explain the difficulty to generate appropriate in vitro glioma models as it prevents compensatory production of αKG.

If this assumption is correct, it provides 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) [93,94] and chloroquine (an anti-malaria drug) [95,96]. As both compounds have

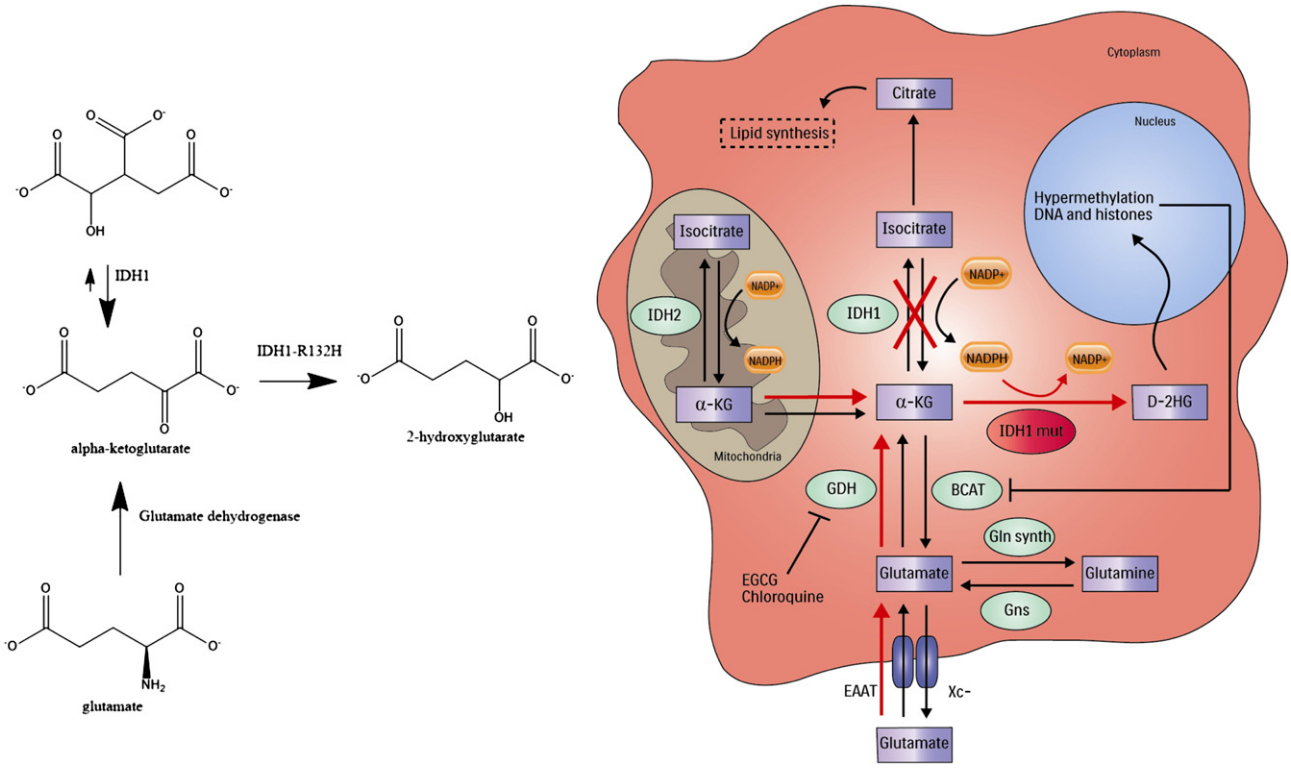


**Fig. 3.** Postulated model of effects of IDH1 mutations on tumor cell metabolism. Pathways which may rescue metabolic defects in IDH1-mutated tumors are depicted as red arrows. Mutant IDH1 reduces αKG to D-2HG. Since the cytosolic αKG pool is depleted, and this metabolite is essential for tumor cell proliferation, compensatory mechanisms must occur. First, αKG which is formed in the mitochondria by IDH2 can be transported to the cytosol via the malate–αKG antiporter. Additionally, glutamate may be imported from the microenvironment via the EAAT2 glutamate importer which is expressed in low-grade glioma. Glutamate can then be converted to αKG by GDH.

additional anti-tumor properties by increasing oxidative stress and inhibiting autophagy [97], studies of their putative anti-glioma activity are warranted.

### 1.11. Glutamate in high-grade glioma

Could inhibition of the conversion of glutamate to αKG be effective in the treatment of primary high-grade glioma which do not carry *IDH* mutations? This is difficult to predict since glutamate metabolism is diametrically different in these tumors. As discussed, IDH wild-type glioma overexpresses BCAT1, and expression of mutant IDH and BCAT1 is mutually exclusive [5]. Downregulation of BCAT1 results in decreased cell proliferation and migration, implying an important function for this enzyme. BCAT1 transfers an amino group from branched chain amino acids to αKG, generating glutamate. Thus, in contrast to low-grade diffuse glioma which, according to the proposed hypothesis, needs to import glutamate from the tumor interstitium, high-grade glioblastoma cells, at least the BCAT1-positive ones, generate their own glutamate. In line with this reasoning, these tumors lack EAAT glutamate importers [9] but instead express high levels of the cystine–glutamate antiporter (System $X_c^-$) [98] resulting in secretion of glutamate into the interstitium in exchange for cystine. Imported cystine aids in resisting oxidative stress in GBM cells whereas glutamate concentrations in the interstitium may reach neurotoxic levels. It has been proposed that this phenomenon leads to neuronal dysfunction or even loss, generating space for GBM cells and simultaneously inducing seizures in these patients [9].

As primary GBMs generate their own glutamate, it is unlikely that interstitial glutamate in the neuropil acts as chemotactic factor for IDHwt glioma cells. Interestingly yet, glutamate has been shown to be implicated in migration of high grade glioma cells via autocrine activation of the $Ca^{2+}$-dependent α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) [99]. Inhibition of the cystine–glutamate antiporter by compounds such as sulfasalazine may therefore have anti-tumor effects on different levels: it will 1) reduce import of cystine and increase oxidative stress in the GBM cells; 2) prevent secretion of glutamate and excitotoxicity, and 3) prevent AMPA-R-stimulated migration [99,100].

With respect to glutamate metabolism it may be argued that in both IDH-mutated and IDH wild-type, BCAT1-positive glioma, cellular αKG levels are continuously depleted, either by conversion to D-2HG or by conversion to glutamate. Whether glutamate is converted back to αKG in BCAT1-expressing glioma is unknown. If so, inhibition of glutamate dehydrogenase may have therapeutic effects in primary high grade glioma as well.

All these questions call for structured and detailed analysis of the responses of IDH-mutated and IDH wild-type glioma to combinations of inhibitors of glutamate dehydrogenase and System $X_c^-$, adjuvantly to standard treatment using Temozolomide and irradiation.

### 1.12. Therapeutic inhibition of mutant IDH

Recently, specific inhibitors of $IDH1^{R132H}$ have been developed which inhibit growth of subcutaneously implanted $IDH1^{R132H}$-mutated glioma xenografts [101]. Whether these inhibitors are effective in orthotopic models of IDH-mutated glioma (under conditions in which a clinically relevant, glutamate-rich tumor microenvironment is mimicked) remains to be tested. In fact, considering the fact that *IDH* mutations impose metabolic stress on glioma cells once neoplastic transformation has occurred, a relieve of this metabolic stress by direct inhibition of $IDH1^{R132H}$ may increase malignancy, rather than inhibit tumor progression.

### 1.13. IDH mutations in the context of other tumor types

Does this proposed model, pointing towards glutamate as a central metabolite fit with the other tumor types carrying *IDH* mutations?

Apart from glioma, *IDH* mutations are found relatively frequently in AML, chondrosarcoma and angioimmunoblastic T cell lymphoma. All these types of cancer have in common that they do not depend on angiogenesis. Glioma resides in the brain parenchyma, the tissue with the highest vascular density in our body and, as discussed earlier, these tumors can progress without neo-angiogenesis [22,23,34]. Especially in low-grade glioma angiogenesis is absent, possibly due to increased activity of prolyl hydroxylases in D-2HG-producing tumors and reduced induction of hypoxia-inducible genes including that of VEGF-A [7].

Chondrosarcomas are poorly vascularized tumors, and AML probably does not require angiogenesis. For AML and lymphoma, a dependency of glutamate may not be a limiting factor for growth as these tumors have access to all necessary building blocks and precursors in the blood. All in all, the effect of *IDH* mutations on tumor biology may be tumor-specific and strongly depend on the tumor microenvironment.

## 2. Conclusion

The discovery of *IDH* mutations in tumor types such as glioma has coincided with the renewed interest in tumor metabolism and metabolic targeting in the last five years. It is clear that during gliomagenesis, a very complex network of metabolic processes evolves which is the result of adaptation of tumor cells to overcome continuous metabolic stress. Finding out which enzymes are central to this adaptation process may reveal Achilles' heels of these devastating tumors. Most interesting, this conceptual shift may result in future therapies of glioma patients with safe, relatively inexpensive and already approved inhibitors, preventing overtreatment with poorly effective and toxic chemotherapies. The availability of orthotopic glioma xenograft models carrying *IDH1*-mutations will certainly be very helpful to design and optimize cellular metabolism-targeting approaches. The challenge for the future will be the identification of the group of patients eligible for these treatment modalities.

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