Mutation of the isocitrate-dehydrogenase (IDH) enzymes is one of the central research topics regarding gliomagenesis. Indeed, 70% of gliomas are associated with a gain-of-function IDH mutation and consequently synthesize the oncometabolite, 2-hydroxyglutarate (2-HG). This review aims to elucidate the effects of 2-HG on gliomagenesis. 2-HG promotes tumorigenesis by impacting metabolism, vascularization and altering the epigenome of glioma cells. Glioma metabolism and vascularization is altered by 2-HG's effect on the stability of hypoxia-inducible factor (HIF) and inhibition of endostatin. However, 2-HG's impacts on epigenetic mechanisms are more profound to gliomagenesis. Through competitive inhibition of JHDMs and TET proteins, 2-HG orchestrates histone and DNA hypermethylation, which is associated with gene silencing and dedifferentiation of cells. The hypermethylator phenotype induced by 2-HG also results in alterations of the interaction of the immune system with the tumour. Additionally, this study reviews 2-HG promotion of tumorigenesis by inhibiting repair of DNA alkylation damage through competitive inhibition of AlkB proteins.

Key words: glioblastoma, cancer metabolism, epigenome, IDH1 mutation, 2-hydroxyglutarate.

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Review paper

The effects of 2-hydroxyglutarate on the tumorigenesis of gliomas

Cara Reiter-Brennan, Lukas Semmler, Andreas Klein

Institute of Biochemistry, Charité – Universitätsmedizin Berlin, Berlin, Germany

Introduction

Gliomas, tumors derived from neural stem cells or glial progenitor cells, are a heterogenous group of brain tumors [1]. They are the most common type brain tumor, representing 50% of all brain cancers [2]. Median survival time for World Health Organization (WHO) classified grade IV malignant gliomas is 15 months, even with aggressive treatment [3, 4]. Glioma research was revolutionized in 2008, when the genetic sequencing of glioblastomas revealed that the mutation of isocitrate dehydrogenase 1 and 2 (IDH1/2) is a prevalent mutation in various glioma entities [5]. The most common *IDH1* mutation is R132H, while the most prevalent *IDH*2 mutations are: R172G, R172K, and R172M [6]. IDH1/2 catalyze the reaction isocitrate and NADP+ to α-ketoglutarate (α-KG) and NADPH+H. The gain of function *IDH1/2* mutations however allow the enzymes to reduce α-KG to the oncometabolite, 2-hydroxyglutarate (2-HG) [7]. In the last years, 2-HG has become the center of research, as this oncometabolite changes the tumor metabolism and alters the glioma epigenome [7, 8]. Different glioma entities have different rates of *IDH1* mutations. The most common *IDH1* mutated gliomas are anaplastic oligodendrogliomas WHO grade III and anaplastic astrocytomas WHO grade III with a 81.6% and 82% *IDH1* mutation rate respectively [9]. However, only 3.1% of gliomas carry *IDH2* mutations [9]. Interestingly, it was also established that *IDH1* and *IDH2* mutations are mutually exclusive in gliomas; barely any gliomas harbor both, *IDH1* and *IDH2* mutations [9]. This indicates at the severity of *IDH1/2* mutation, as only one mutation is enough to mediate tumorigenesis.

The relevance of *IDH1/2* mutations is also demonstrated by the WHO classification system, which was updated in 2016 to accommodate the genetic makeup of the different glioma subtypes. Relevant to this review is the division between low-grade gliomas (LGG) (grade I and II) and grade III and IV gliomas, also known as high-grade gliomas [10]. Whereas grade I gliomas are benign, grade IV tumors, also known as *IDH*-mutant or *IDH*-wild-type glioblastoma, are refractory to chemotherapy and show more advanced characteristics of malignancy [11]. Grade II gliomas almost universally transform to high-grade gliomas [12]. Regarding grade II–IV gliomas, the updated WHO classification of 2016 subdivides lesions according to molecular markers, such as the presence of *IDH* mutations or 1p/19q deletion [10]. Of importance for this review is also the differentiation between primary and secondary glioblastomas. While primary glioblastomas are *de-novo* tumors, secondary glioblastomas develop from low-grade diffuse astrocytomas or anaplastic astrocytoma [13]. The distinctive genetic marker of secondary glioblastomas is the *IDH1* mutation, as over 80% of secondary glioblastomas illustrate *IDH1* mutations compared to only 5% of primary glioblastomas [14]. Secondary glioblastomas are also associated with a hypermethylator phenotype and exhibit a better prognosis than primary glioblastomas [14]. Due to the strong association with *IDH1* mutations, this review will focus particularly on the pathogenesis of secondary glioblastomas.

2-hydroxyglutarate

IDH1/2 mutations are one of the earliest known mutations occurring during glioma formation [15]. When measuring the catalytic activity of mutant IDH1, Dang *et al.* [7] observed a "1,000 fold decrease in catalytic turnover" from isocitrate to α-KG. Conversely, studies suggest that cancers harboring *IDH1/2* mutations produced 2-HG concentrations 10 to 100 times the levels of cancers with wild type IDH [16]. Further, while wild-type IDH1/2 produces the reducing agent NADPH+H+ during the reaction isocitrate to α-KG, mutant IDH1/2 consumes NADPH+H+ during the catalyzation of α-KG to 2-HG [7] (Fig. 1). This is significant, as NADPH+H+ is an important metabolite for macromolecule synthesis and defending cells against reactive oxygen species [17]. *IDH1/2* mutations could offset the cellular redox reactions, promoting tumorigenesis [17]. 2-HG is a chiral molecule, so D- and L-enantiomer forms exist (Fig. 1). *In vivo* and *in vitro* experiments established that mutant IDH1/2 produces almost exclusively D-2-HG [16]. Therefore, D-2-HG seems to be the enantiomer most relevant to tumorigenesis. Intriguingly, while cancer cells produce almost only D-2-HG, numerous studies observed that *in vitro*, L-2-HG is a more potent inhibitor of various enzymes and has a greater effect on cell proliferation [18–20]. While *IDH1/2* mutations have a variety of consequences on tumorigenesis, the correlation between remarkably high levels of 2-HG in gliomas led to much investigation of the specific effect of 2-HG on gliomagenesis.

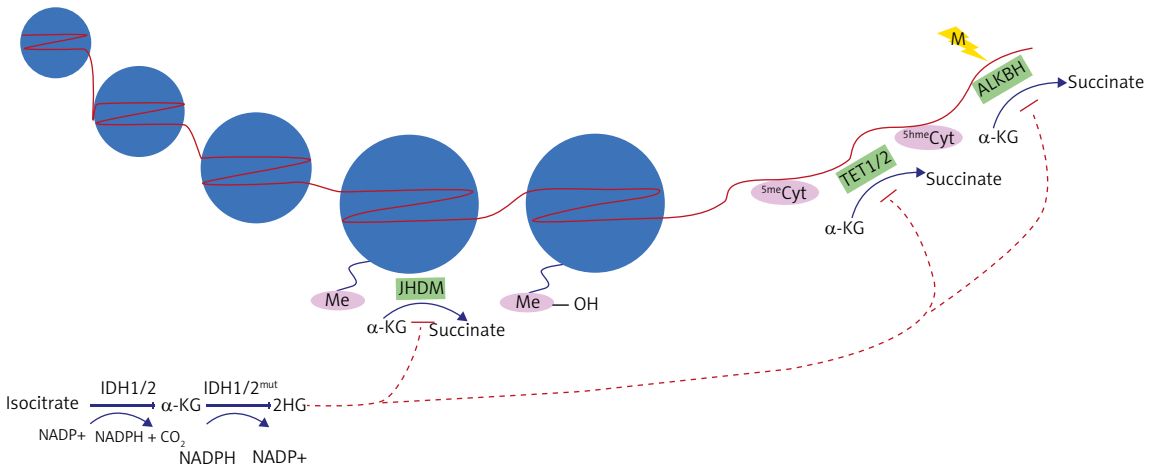
α-ketoglutarate dependent dioxygenases

Vital to the understanding of 2-HG's role in tumorigenesis is the consideration of 2-HG's similar structure compared to α-KG. The keto group of α-KG is simply exchanged for a hydroxyl group in 2-HG [21]. Due to the structural similarity of both metabolites, 2-HG acts as a competitive inhibitor to α-KG dependent dioxygenases [18]. There are over 60 known α-KG dependent dioxygenases which could potentially be inhibited by 2-HG [22]. Especially relevant to cancerogenesis are the α-KG dependent dioxygenases which catalyze hydroxylation reactions, such as DNA demethylation, histone demethylation, sensing of hypoxia and DNA repair of alkylation damage [22, 23]. For α-KG dependent enzymes, 2-HG acts as a competitive inhibitor and can thereby influence glioma tumorigenesis.

Hypoxia-inducible factors

α-KG dependent dioxygenases also play an important role during O_2-sensing in cells. Hypoxia-inducible transcription factors (HIF 1–3) coordinates the body's response to hypoxia, ranging from the formation of new blood vessels to increased synthesis of red blood cells [24]. HIF-α is degraded through post-translational hydroxylation, catalyzed by prolyl hydroxylase (also called EGLN), a α-KG dependent dioxygenase [22]. All α-KG dependent dioxygenases require oxygen as a co-substrate. In the case of HIF-α, if oxygen is present, prolyl hydroxylase can hydroxylate the prolyl residues on the HIF-α subunit. Von Hippel-Lindau protein can now bind to the hydroxylated HIF-α subunit, which leads to ubiquitination and the breakdown of HIF-α in proteasomes [22]. There is conflicting research regarding the impact of 2-HG on HIF.

On one hand, research has shown that 2-HG inhibits prolyl hydroxylase. A study conducted by Xu *et al.* [18] illustrated that *in vivo*, HIF-α1 levels increased after the knockdown of wild type *IDH*, introduction of mutant *IDH* or



IDH1/2 – isocitrate-dehydrogenase-1/2; α-KG – α-ketoglutarate; 2-HG – 2-hydroxyglutarate; JDHM – Jumonji C domain-containing histone demethylase; Me – methylated lysin residue on histone; TET1/2 – ten-eleven translocation enzymes 1/2; ^{5me}Cyt – 5-methylcytosine; ^{5hme}Cyt – 5-hydroxymethylcytosine; ALKBH – α-KG-dependent alkB homolog; M – methylation-damage

Fig. 1. In physiological conditions, IDH1/2 metabolizes isocitrate to α-KG with the production of NADPH and CO_2. Cancerous mutated IDH1/2 synthesizes 2-HG and NADP+ from α-KG. 2-HG competitively inhibits numerous α-KG-dependent-dixoxygenases. Here illustrated is 2-HG's competitive inhibition of JHDMs and TET1/2, which results in histone and DNA methylation, respectively. 2-HG also inhibits ALKBHs, leading to less DNA repair of methylation damage. Overall, these effects induced by 2-HG alter the epigenome of glioma cells and promote tumorigenesis

addition of 2-HG. Other research groups also noticed the correlation between *IDH1/2* mutations and elevated HIF-1α expression [25–27]. Interestingly, it was also observed that L-2-HG is a more potent competitive inhibitor than D-2-HG [18]. This would make sense, as it has been reported that L-2-HG is synthesized primarily by cells in hypoxic microenvironments and oxygen starved tissue has a special need for angiogenesis to combat hypoxia [28]. Under these physiological hypoxic conditions, L-2-HG is produced predominantly by lactatedehydrogenase A (LDHA) and to a lesser extent by malatedehydrogenase 1 and 2 (MDH1/2) (Fig. 2) [28]. Nonetheless**,** both enantiomers stabilize HIF-α through competitive inhibition of prolyl hydroxylase and thereby promote tumorigenesis. This is because the same mechanisms that allow cells to survive during hypoxia help tumor growth, as HIF regulated genes increase angiogenic factors, glycolysis, glucose transporters, invasion factors and survival factors [29].

On the other hand, research also suggests that D-2-HG promotes HIF-1α degradation by stimulating prolyl hydroxylase activity [19]. Koivunen *et al.* [19] obtained similar results as the papers mentioned above in regard to L-2-HG, as they observed that L-2-HG inhibited prolyl hydroxylase. Surprisingly however, this research group also describes that instead of inhibiting, D-2-HG potentiated prolyl hydroxylase catalytic activity, resulting in HIF-1α degradation. The paper reports that HIF-1α levels were reduced in human *IDH*-mutated proneural tumors, compared to *IDH*-wild type samples [19]. Other research groups also concluded that the addition of D-2-HG but not L-2-HG, reduced HIF-1α concentration in cells and that *IDH* mutation was not sufficient to upregulate HIF-1α concentrations in gliomas [30, 31]. Supporting, but not proving the higher prolyl 4-hydroxylase activity, *IDH*-mutated cancers are associated with heightened expressions of prolyl 4-hydroxylases, which tag HIF-1α for ubiquitination and subsequent proteasomal degradation [32]. The consequence of this mechanism on tumor growth was also tested, as D-2-HG stimulates activity of prolyl 4-hydroxylase and subsequent HIF-1α degradation which reduced colony formation of immortalized human astrocytes [19].

Concluding, on the one hand 2-HG was associated with HIF-1 stabilisation while other studies have also reported that D-2-HG induces HIF-1α degradation. In part, these conflicting results can be resolved by the discovery that D- and L-2-HG suppress factor-inhibiting HIF1 (FIH1) [33, 34]. If FIH1 suppresses activation of HIF-1α, then an inhibition of FIH1 through 2-HG is an alternative mechanism which can lead to HIF-1α stabilisation [33, 34]. Nevertheless, the opposing results show that the 2-HG impact on HIF-1α has to be clarified.

Endostatin

Unlike HIF, endostatin is a natural inhibitor of angiogenesis, which reduces tumor vascularization and suppresses tumor growth [35]. Upregulation of tumor vascularization is an important step during gliomagenesis, as glioblastomas are characterized by extensive angiogenesis [36]. Further, neovascularization is associated with higher malignancy grade and reduced post-operative survival in patients suffering from gliomas [36]. Similarly to HIF, endostatin synthesis is also affected by 2-HG accumulation in *IDH*-mutated gliomas. Endostatin is synthesized through prolyl-hydroxylation of collagen prolyl 4-hydroxylase [35]. As prolyl 4-hydroxylase is α-KG dependent, researchers postulate that 2-HG can competitively inhibit this enzyme, leading to lower concentrations of endostatin in *IDH*-mutated gliomas [37]. In fact, *IDH*-mutated gliomas were associated with reduced levels of endostatin and higher rates of blood vessel densities [37]. The effect of 2-HG on endostatin was more directly confirmed by the observation that 2-HG injection into cells decreased endostatin levels [18]. Thereby, 2-HG promotes tumorigenesis by inhibiting endostatin, which then leads to tumor vascularization and proliferation.

Epigenetics during cancerogenesis

While traditionally, 2-HG's impacts on gliomagenesis have been associated with alterations to tumor metabolism and vascularization, more recent research suggests that glioma formation is driven to a greater extent by epigenetic alterations induced by 2-HG. In essence, epigenetics describes modifications of chromatin structure which consequently alter gene expression. The chromatin configuration influences whether DNA transcription machinery can access DNA to facilitate transcription [38]. Regarding gliomagenesis, the two most examined epigenetic mechanisms altering chromatin structure are DNA methylation and histone modification [39]. Depending on the histone methylated, chromatin structure is loosened to promote transcription, or tightened to suppress transcriptional activities. For instance, trimethylation of lysin 4 on histone 3 (H3K4me3) is associated with genes that are transcriptionally active, while trimethylation of H3K9 (H3K9me3) at gene promoters is one of the chief epigenetic silencing mechanisms in mammalian cells [39]. DNA methylation occurs on the GpG Islands of the genome; these are repetitive sequences of DNA and comprise 60% of the promoters [40]. While histone modifications can either enhance or repress transcription, DNA methylation leads to gene silencing [39]. Adding to the complexity of epigenetic mechanisms, DNA methylation can impact histone methylation and vice versa. While DNA methylation and histone methylation are often described as independent entities, they can interact with each other to modify cellular transcription status [39].

α-KG dependent histone demethylase

In 2009, Tsukada *et al.* [41] discovered a group of histone demethylases named Jumonji C (JmjC) domain-containing histone demethylase (JHDM), which in presence of α-KG and iron, demethylate lysin residues on histones. JHDMs are also known as Lysin (K)-specific demethylases. This group contains a variety of JHDMs, which demethylate different lysin residues. Due to 2-HG's similar structure to α-KG, 2-HG competitively inhibits JHDMs (Fig. 2). The three most important JHDMs during gliomagenesis are JHDM1A (official symbol KDM2A), JMJD2A (KDM4A), and JMJD2C

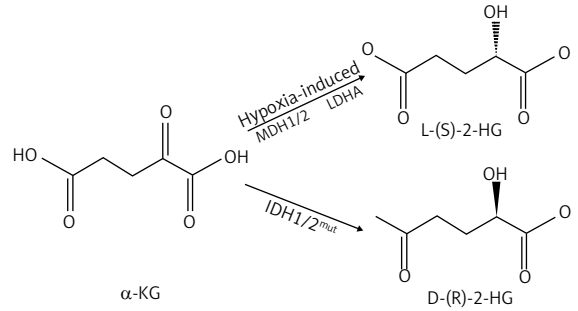


Fig. 2. 2-hydroxyglutarate (2-HG) is a chiral molecule, which exists in a L-(S)-2-HG and D-(R)-2-HG form. In cancers, mutated IDH1/2 generates almost exclusively D-(R)-2-HG from α-ketoglutarate (α-KG). L-(S)-2-HG is synthesised during hypoxic conditions through lactate-dehydrogenase A (LDHA) and malatedehydrogenase (MDH1/2)

(KDM4C). Xu *et al.* [18] reported that elevated 2-HG levels from mutant IDH1 can inhibit multiple α-KG dependent histone demethylases *in vivo* and *in vitro*. As seen in other α-KG dependent dioxygenases, L-2-HG was found to be significantly more potent than D-2-HG [18, 42]. The effect of 2-HG on histone methylation was further demonstrated *in vivo* by the observation that *IDH*-mutated glioma samples with abnormally high 2-HG levels exhibited increased methylation marks of the following histones: H3K4, H3K9, H3K27 and H3K79 [18]. This observation was supported by other research groups as well [43]. The methylation of histones explored by these papers do not all orchestrate the same epigenetic effects; mono-methylation of H3K4, H3K27 and H3K79 are associated with gene activation, while trimethylation of H3K9, H3K27 and H3K79 suppresses transcription [44]. Current research is especially focused on the trimethylation of H3K9 (H3K9me3). While H3K9me3 has long been known as an epigenetic silencing mechanism, it also plays an important role during tumorigenesis. H3K9me3 inhibits transcription and promotes heterochromatin formation [45]. Intriguingly, H3K9me3 is associated with *IDH1* mutation in different types of gliomas [43, 46, 47]. Supporting this observation, *in vitro* experiments on cells of the central nervous system showed that *IDH*-mutated astrocytes or murine neurosphere cultures exhibited enhanced H3K9me3 [43, 47]. Evidence further suggests that other mechanisms of histone methylation, other than inhibition of JHDMs, are important for gliomagenesis. The methyltransferase, Enhancer-of-zeste homolog 2 (EZH2), which catalyzes the methylation of H3K27, is overexpressed in cancers such as lung, breast, prostate, blood cancers and glioblastomas [48]. EZH2 was also found to be vital in glioblastoma cells to maintain stem-cell-like properties and therefore promote tumorigenesis [49]. This suggests that histone methylation in general is an important epigenetic mechanism orchestrating carcinogenesis, which delineates the importance of 2-HG induced histone methylation for glioma formation. The significance of histone methylation to tumorigenesis is also evident from much research showing that histone methylation inhibits cellular differentiation. For instance, a study reported that the "introduction of mutant but not wild-type *IDH1* into astrocytes resulted in the upregulation of nestin (and other genes associated with stem cell identity) at the time of DNA methylation increase and the adoption of a neurosphere/stem-like phenotype" [43]. Regarding gliomas, *IDH*-mutated gliomas with methylated H3K9 exhibit lower levels of glial fibrillary acid protein (a protein important for central nervous system cell differentiation) and less cellular differentiation [47]. Moreover, the cancerogenic effect of histone methylation is supported by the consideration that histone methylation can impact DNA methylation [46]. For instance, Turcan *et al.* [43] observed that specifically H3K9me3 is associated with DNA methylation, which could in turn promote cellular proliferation.

Ten-eleven translocation enzymes

DNA methylation is an important characteristic of tumorigenesis, as cancer cells often exhibit atypical DNA methylation patterns, including global DNA hypomethylation and promoter hypermethylation [50]. Both effects are pro-tumorigenic, as hypomethylation leads to genome instability, while promoter hypermethylation, amongst other things, silences tumor suppressor genes [50]. While DNA methylation is usually catalyzed by DNA-methyl-transferases, de-methylation is initiated by the three ten-eleven translocation enzymes (TETs 1/2/3) [51]. TETs are another type of α-KG dependent dioxygenases [51]. De-methylation occurs in a two-step process. First, TET enzymes successively oxidize 5-methylcytosine to 5-hydroxymethylcytosine (5hmC), to 5-formylcytosine (5fC) and finally to 5-carboxylcytosine (5caC). The three oxidized methyl-cytosines, 5hmC, 5fC and 5caC, are collectively coined "oxi-methyl-cytosines (oxi-mCs)". Next, the modified cytosines of 5fC and 5caC are exchanged for non-methylated cytosines through base excision, thereby restoring the un-methylated cytosine. Out of the three oxi-mCs, 5hmC is the most prevalent because only 1-10% of 5hmCs are further oxidized to 5fC and 5aC [52]. This is why 5hmC is often used as a marker to measure TET-enzyme activity. Explored more extensively in the following reviews, 5hmC is an important metabolite that contributes to cancer development [53–55].

In fact, TET2 is described as a tumor suppressor because studies suggest that many solid cancers and 15% of myeloid cancers carry TET2 mutations [56–58]. The catalytic activity of TET enzymes is dependent on α-KG, thereby researchers hypothesized that 2-HG from *IDH*-mutated gliomas competitively inhibits TET proteins. In a study by Xu *et al.* [18], D-2-HG inhibited TET1 up to 47% and TET2 up to 83%. Similar to the inhibition of prolyl hydroxylase, L-2-HG was more potent than D-2-HG. As expected from inhibition of a demethylating enzyme, high 2-HG and inhibition of TET de-methylation activity was associated with DNA hypermethylation (Fig. 2) [18]. Xu *et al.* [18] demonstrated that *in vivo*, 5-hmC was much lower in mutated *IDH1* glioma samples compared to wild-type *IDH1*, indicating the impaired function of TET to demethylase DNA in the presence of abnormally high 2-HG levels. These findings are supported by observations that *IDH*-mutated acute myeloid leukemia cells showed impaired TET2 functions and hypermethylated phenotypes [21]. Not only is there

a clear correlation between 2-HG dependent TET inhibition and DNA hypermethylation, but there is also evidence that 2-HG impairment of TET enzymes induces cancer formation. 2-HG induced hypermethylation silences tumor suppressors and inhibits cellular differentiation [43, 59–61]. Turcan *et al.* [43] observed that the introduction of mutant *IDH1* into astrocytes coincided with increasing DNA methylation and the adoption of a stem-cell-like phenotype. Another study compared *TET2* mutant mice to *IDH*-mutant mice exhibiting high levels of 2-HG. Both types, *TET2* mutant mice and *IDH*-mutant mice, showed "stem cell expansion and impaired hematopoietic differentiation" [60]. Finally, in a very recent study, induction of TET2 expression in glioblastoma cells upregulated genes associated with differentiation of neural cells, such as the brain fatty acid-binding protein [61]. This suggests that inhibition of TET2 through 2-HG could impair nerve cell differentiation.

Immune system

The relationship between cancer and the immune system is another important factor driving tumorigenesis. Cancer cells apply various methods to circumvent host immune surveillance in order to proliferate.

Current research provides evidence of novel immune evasive mechanisms specific to *IDH*-mutated gliomas. Using orthotopic syngeneic low-grade glioma models, Kohanbasch *et al.* [62] discovered that 2-HG reduces the expression of *STAT1*. STAT1 is a regulator of the chemokine, CXCL10, which in turn attracts CD8+ cytotoxic T-cells. Thereby, a 2-HG induced abatement of STAT1 reduces CD8+ T-cell accumulation at the tumor site. When implanting *IDH*-mutated low-grade glioma cell lines in syngeneic mice, the developing tumors showed lower rates of CXCL10 and less CD8+ T-Cell infiltration than tumors from *IDH*-wild type cells. The effects of 2-HG were directly tested, as inhibiting 2-HG synthesis in *IDH*-mutated glioma cell lines resulted in an increase in CXCL10 levels and elevated expression of STAT1. Conversely, treatment of *IDH*-wild type glioma cells lines with 2-HG resulted in reduced STAT1 expression [62]. A supplementary review of these findings can be found in the commentary by Lucca and Hafler [63]. Kohanbasch's *et al.* conclusion is supported by another study revealing a negative correlation between pathological glioma grade and STAT1 gene expression [64]. Overall, healthy brain tissue shows significantly higher average expression of STAT1 than glioma tissue [64].

However, evidence suggest that rather than stimulating immunoevasion of glioma cells, 2-HG reduces the immunosuppression usually associated with cancer and thereby impedes cancerous cell proliferation. *IDH*-mutated gliomas are associated with a more active immune system than non-*IDH*-mutated gliomas [65, 66]. Studies observed that *IDH*-wild type gliomas show a greater infiltration of immunosuppressive cells, such as T-regulatory cells (T-Regs) and tumor-associated macrophages (TAMs), than *IDH*-mutant gliomas [65–67]. T-Regs infiltrate tumor tissue and inhibit T-cell toxicity while simultaneously suppressing tumor antigen presentation by dendritic cells [68]. Moreover, while macrophages usually exhibit anti-tumorigenic functions, in response to the local tumor microenvironment, TAMs alter their function and facilitate tumor growth instead [68]. This conclusion is supported by a recent study by Rahman *et al.* [69], who investigated expression of immunogenic markers in *IDH*-mutated glioblastomas. CD163, a member of the scavenger receptor of the cysteine-rich (SRCR) superfamily, is a marker for M2 Macrophages, which are a subtype of anti-inflammatory TAMs [70]. *IDH*-mutated glioblastomas are associated with a significantly lower expression of CD163, which could reduce immunosuppression and suppress tumor growth [69]. For instance, CD163-positive cancer cells are potentially associated with a higher malignant potential in clear cell renal cell carcinoma [71]. In the tumor microenvironment, CD70, member of the tumor necrosis factor (TNF) family, functions as an immunosuppressive factor [72]. CD70 expression is also decreased in *IDH*-mutated glioblastomas, resulting in more anti-tumor immunoactivity [69]. This difference in tumor immune cell infiltration is particularly evident when comparing secondary glioblastomas to primary glioblastomas. Amankulor *et al.* [66] explored the reasons why secondary glioblastomas tumors exhibit less immunosuppressive tumor-associated cell infiltration than primary glioblastomas. They posit that, through mechanisms not quite clear, 2-HG increases methylation and thereby silences genes associated with the tumor-derived immune system. Consequently, *IDH*-mutated gliomas with high levels of 2-HG exhibit less immunosuppressive cell infiltration and a more active host immune system. As a result, the host immune system can induce an anti-tumor immune response, inhibiting tumorigenesis. Amankulor *et al.* postulate that the milder cancer progression of secondary glioblastoma patients is partly related to patients high 2-HG levels, which translate into a more immune-active phenotype that prohibits cancer proliferation [66]. Additionally, some studies reported that through 2-HG induced promoter methylation, *IDH*-mutated gliomas illustrate a lower expression of the transmembrane protein, programmed death ligand-1 (PD-L1), than *IDH*-wild type glioma cells [65, 67]. PD-L1, expressed on cancer cells, binds to the programmed death receptor-1 on T-cells, and consequently inhibits T-cell immunoactivity and leads to immunosuppression [73]. However, PD-L1 is only amplified in 0.3% of glioblastomas [74]. Thus, glioblastomas do not rely on PD-L1 as their main immunoevasive strategy.

AlkB homolog, α-ketoglutarate-dependent dioxygenase

Cancer cells steadily have to adapt to a changing environment. Therefore, elevated mutation rates in cancer cells increase the genetic diversity of the tumor and allow cancer cells to express a phenotype which is best suited to their environment. Thus, it can be argued that DNA mutations within existing cancerous tissue can promote tumorigenesis [75]. These DNA mutations can result from methylation of DNA bases through environmental or endogenous alkylating agents. The described microevolution of cancer is suppressed if DNA mutations, such as alkylated DNA bases, are repaired. In mammalian cells, DNA al-

kylation is corrected by the de-alkylating enzymes, AlkB proteins, which remove methyl groups from 1-methyl-adenine and 3-methyl-cytosine [76, 77].

AlkB proteins are α-KG and iron dependent di-oxygenases [76]. Wang *et al.* [77] observed that D-2-HG inhibits the repair of methyl-adenine by ALKBH2 and ALKBH3 *in vitro* (Fig. 2). They point out that D-2-HG is a weak inhibitor of ALKBH at physiological concentrations. However in higher concentrations as of 0.5 mM, D-2-HG results in a 50% inhibition of ALKBH2 [77]. Xu *et al.* [18] support this hypothesis, as they write that "the molar ratio of *R*-2-HG (D-2-HG) and α-KG has been calculated as an average 373-fold in glioma patients, enabling *R*-2-HG (D-2-HG) to exert its inhibitory effects". From this it can be hypothesized that D-2-HG's competitive inhibition of ALKBH elevates intra-cancerous mutation rate and promotes glioma microevolution.

Intriguingly however, the inhibition of DNA repair mechanisms through D-2-HG enhances the effectiveness of chemotherapy. Wang *et al.* [77] observed that *IDH1/2* mutated glioma cells are more sensitive to alkylating chemotherapy and this sensitivity was abolished when D-2-HG production was disrupted. Another study explored the performance of the alkylating chemotherapy, PCV (procarbazine, lomustine and vincristine), on patients with *IDH*-mutated anaplastic oligodendrogliomas and *IDH*-wild type anaplastic oligoastrocytomas [78]. Here, patients with *IDH* mutations responded better to the alkylating agents than patients with wild-type IDH [78]. The study concludes that screening patients for *IDH* mutations is beneficial to decipher if they would benefit from neo-adjuvant PCV treatment [78]. In another paper, the overexpression of ALKBH2 in glioblastomas resulted in a resistance to the alkylating agent, temozolomide. Knockdown of ALKBH2 increased sensitivity to temozolomide [79].

Conclusions

Ultimately, the oncometabolite 2-HG promotes cellular cancerogenesis in gliomas. While tumor metabolism and angiogenesis are also affected by 2-HG, epigenetic consequences stemming from the 2-HG induced hypermethylator phenotype, especially associated with secondary glioblastomas, are chiefly responsible for tumor progression. Histone and DNA methylation promote gene silencing and lead to cellular dedifferentiation. A prominent example of the epigenetic alterations induced by 2-HG are the impacts on the immune system. However, this review has also elucidated some controversies in current research. Some argue that 2-HG does not directly promote tumorigenesis, as certain publications observed 2-HG induced destabilization of HIF and reduction of immunosuppressive cell infiltration. However, on the whole, these anti-tumorigenic effects of 2-HG are largely dispensable, because it seems they only translate into a slightly milder tumor progression of secondary glioblastoma patients; gliomagenesis occurs nonetheless. While the rough outlines of 2-HGs effects on gliomagenesis are becoming clearer, more extensive research is necessary to fully elucidate the pathogenesis of *IDH*-mutated gliomas. Especially the impact of epigenome alterations and the interactions between histone and DNA methylation is still vague. Understanding the pathogenesis of gliomas is imperative in order to generate effective therapies for *IDH*-mutated gliomas.

The authors declare no conflict of interest.

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Address for correspondence

Cara Reiter-Brennan
Institute of Biochemistry
Charité – Universitätsmedizin Berlin
Charitéplatz 1
10117 Berlin, Germany
e-mail: cara.reiter-brennan@charite.de

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