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.

**Introduction**

Gliomas, tumors derived from neural stem cells or glial progenitor cells, are a heterogeneous 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 IDH2 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 catalysis 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 O2-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

Fig. 1. In physiological conditions, IDH1/2 metabolizes isocitrate to α-KG with the production of NADPH and CO2. Cancerous mutated IDH1/2 synthesizes 2-HG and NADP+ from α-KG. 2-HG competitively inhibits numerous α-KG-dependent-dioxygenases. 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 lactate dehydrogenase A (LDH-A) and to a lesser extent by malate dehydrogenase 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 stabilization 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 further 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 lysine 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 CpG 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 (JmC) 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), JMID2A (KDM4A), and JMID2C.
L-(S)-2-HG is synthesised during hypoxic conditions through lactate-dehydrogenase A (LDHA) and malate-dehydrogenase (MDH1/2).

Intriguingly, H3K9me3 is associated with histone methylation to tumorigenesis. While H3K9me3 inhibits transcription and promotes heterochromatin formation, it also plays an important role during tumorigenesis. The methylation of H3K9 (H3K9me3) is the most prevalent because only 1–10% of 5hmCs are converted to 5-methylcytosine (5mC). Ten-eleven translocation enzymes (TETs 1/2/3) catalyzes the methylation of 5mC, thereby forming 5-formylcytosine (5fC) and finally to 5-carboxylcytosine (5caC). The three oxidized methylcytosines, 5mC, 5fC and 5caC, are collectively coined as “oxi-methyl-cytosines (oxi-mCs)”. Next, the modified cytosines through base excision, thereby restoring the “oxi-mCs” back to 5-carboxylcytosine (5caC). The three oxidized methylcytosines, 5mC, 5fC and 5caC, are collectively coined “oxi-methyl-cytosines (oxi-mCs)”. Next, the modified cytosines through base excision, thereby restoring the “oxi-mCs” back to 5-carboxylcytosine (5caC). The three oxidized methylcytosines, 5mC, 5fC and 5caC, are collectively coined “oxi-methyl-cytosines (oxi-mCs)”. Next, the modified cytosines through base excision, thereby restoring the un-methylated cytosine. Out of the three oxi-mCs, 5mC is the most prevalent because only 1-10% of 5hmCs are further oxidized to 5fC and 5caC [52]. This is why 5mC 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

![Diagram of histone methylation and DNA demethylation](image-url)
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 IDH1-mutant mice exhibiting high levels of 2-HG. Both types, TET2 mutant mice and IDH1-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 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 suggests 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 immunoreactivity 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-
HIF and reduction of immunosuppressive cell in-cidated some controversies in current research. Some ar-
zon the immune system. However, this review has also elu-
the epigenetic alterations induced by 2-HG are the impacts
lead to cellular dedifferentiation. A prominent example of
Histone and DNA methylation promote gene silencing and
alterations and the interactions between histone and DNA
methylation is still vague. Understanding the pathogene-
sis of gliomas is imperative in order to generate effective
therapies for IDH-mutated gliomas.

Conclusions
Ultimately, the oncometabolite 2-HG promotes cellular
cancerogenesis in gliomas. While tumor metabolism and
angiogenesis are also affected by 2-HG, epigenetic conse-
quences stemming from the 2-HG induced hypermethyla-
tor phenotype, especially associated with secondary glo-
blastomas, 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 elu-
cidated some controversies in current research. Some ar-
gue that 2-HG does not directly promote tumorigenesis, as
certain publications observed 2-HG induced destabil-
ization of HIF and reduction of immunosuppressive cell in-
filtration. 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 re-
search is necessary to fully elucidate the pathogenesis of
IDH-mutated gliomas. Especially the impact of epigenome
altered.
The effects of 2-hydroxyglutarate on the tumorigenesis of gliomas

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