Wild-type isocitrate dehydrogenase under the spotlight in glioblastoma

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INTRODUCTION

GLIOBLASTOMA (GBM) is the most common primary brain tumor in adults, and accounts for more than 2500 cases diagnosed each year in France. This highly malignant and rapidly progressive glioma is distinct histologically from lower-grade tumors by necrosis and hypoxia-induced microvascular hyperplasia. Patients die within 4 months without therapy, while median survival of those receiving radiotherapy with concomitant and adjuvant temozolomide chemotherapy (Stupp protocol) is improved to 15 months [1, 2]. Still, less than 5% of patients survive over 5 years due to invariable GBM relapse [3]. For most patients with GBM, there is no known cause of the disease and no early detection available. Thus, it is essential to better understand the biology of GBM to develop treatment strategies to effectively cure them. One avenue of research that is relatively unexplored in the field of neuro-oncology is how metabolism is rewired in these brain tumors.

Metabolic pathways are core mechanisms that cells use to fuel their growth and survival. One of the major consequences of the genetic and molecular alterations occurring in GBM is an altered cellular metabolism, recognized in fine as a key driver of tumor progression. Besides these distinct intrinsic alterations, extrinsic features such as the tumor microenvironment or exposition to treatments may also disrupt activity of several pathways, resulting in distinct metabolic phenotypes [4]. Recent studies have revealed remarkable metabolic heterogeneity and plasticity among GBM but also within distinct regions of the same tumor. In particular, we and others demonstrate that the same genetic signature, tumor sublocation in hypoxic region as well as stemness features delineate GBM metabolic rewiring [5–8]. This metabolic heterogeneity would explain why cancer cells with different genetic alterations can display similar metabolic phenotypes whereas cancer cells with identical genetic alterations have different metabolism. One hallmark of metabolic reprogramming is highlighted by enhanced aerobic glycolysis along with excess lactate secretion, termed “the Warburg effect”. Advanced analytical techniques through metabolomics, fluxomic isotope tracers, and metabolic imaging show variations of other critical metabolic circuits including glutaminolysis, one-carbon metabolism, lipid and nucleotide synthesis, as well as reactive oxygen species (ROS) management [9]. While the Warburg effect plays an important role in clinical imaging for cancer through PET scan analysis by measuring higher concentrations of radioactive glucose analog in malignant cancers than in other tissues, this technology is not suitable for GBM due to high background signals. Indeed, the brain is the main consumer of glucose in the body but lacks fuel store, and hence requires a continuously huge supply of glucose [10]. Thus, in the particular context of GBM, development of novel radiotracers based on amino acid or lipid metabolism would definitively improve GBM diagnosis and follow-up, which are currently mainly resting on common imaging methodologies such as MRI and CT scans. Furthermore, therapeutic opportunities might arise if we can identify specific metabolic liabilities in GBM cells, distinct from canonical metabolic pathways supporting cell growth of normal cells.

Recent insights in metabolomic studies have suggested a key role of wild-type IDH enzymes upon treatment to favor GBM proliferation and recurrence [11]. The discovery that patients with mutant IDH1/2 GBM have a better outcome compared to those with wild-type enzymes has spurred robust research to study the consequences of IDH mutations on cellular metabolism and to design new effective targeted molecular therapies. Given that wild-type isoforms account for more than 90% of all GBM, it is now time to capitalize on the knowledge built from mutant IDH1/2 targeting to outline rationale
on wild-type IDH enzymes targeting. Here, we provide an overview of the metabolic properties of IDH enzymes and their potential as new therapeutic targets against GBM.

**METABOLIC PROPERTIES OF IDH ENZYMES**

IDH enzymes have been known for decades to catalyze the oxidative decarboxylation of isocitrate producing α-ketoglutarate (αKG) and carbon dioxide (CO₂) while reducing cofactors NAD(P)⁺ to NAD(P)H (Fig. 1). In all eukaryotic cell types except mature red blood cells, three different IDH paralogs exist. IDH1 and IDH2 are homodimeric NADP⁺-dependent enzymes and mostly differ by their localization, IDH1 being cytoplasmic while IDH2 is expressed in mitochondria. In contrast, IDH3, also expressed in the mitochondria, uses NAD⁺ as cofactor, forms heterodimers or heterotetramers composed of αβ and αγ subunits and works in an irreversible manner. These 3 IDH isoforms have overlapping but nonredundant roles in metabolism including, but not limited to, mitochondrial oxidative phosphorylation, glutamine metabolism, lipogenesis, glucose sensing, and regulation of cellular redox status [12, 13] (Fig. 1).

**Canonical functions of IDH enzymes**

*Production of αKG, a mitochondrial key metabolite with pleiotropic activity.*

Mitochondrial metabolites generated through the tricarboxylic acid (TCA) cycle are crucial for the biosynthesis of macromolecules such as nucleotides, lipids, and proteins. TCA cycle is mainly fueled through 2 metabolic inputs, one from glucose-derived pyruvate and the other one from glutamine-derived carbons and carbon dioxide with the production of reducing equivalent NAD(P)H. Whereas this reaction is irreversible through IDH3 within the TCA cycle, IDH1/2 activities are working in a reversible manner.

Through the succinate dehydrogenase and the fumarate hydratase, respectively. In combination with IDH3, IDH2 regulates the TCA cycle running through its ability to work in a reversible manner by converting αKG back to isocitrate (Fig. 1). This cycle is completed by the transfer of electrons from NADH to NADPH through the nicotinamide nucleotide transhydrogenase [15]. Of note, other enzymes, including glutamate-pyruvate transaminases (GPT1/2) and glutamate-oxaloacetate transaminase (GOT1/2), can also produce αKG, allowing parallel synthesis of alanine and aspartate, respectively, that can also be used as precursors for TCA cycle intermediates and protein synthesis.

Besides its critical role in metabolic cellular homeostasis, αKG is also an obligatory cofactor of dioxygenases enzymes, a large group of phylogenetically conserved enzymes including the prolyl-hydroxylase (PHD) and multiple demethylases, which play a key role in important processes such as responses to hypoxia and chromatin modifications respectively. Precisely, αKG regulates PHD activity involved in the stabilization of the hypoxia-inducible factor-1α (HIF-1α), a master regulator of oxygen homeostasis (Fig. 1). Under limited oxygen conditions or reduced levels of αKG, PHD activity is impaired resulting in HIF-1α translocation to the nucleus where it regulates the transcription of genes mainly involved in metabolism, erythropoiesis, and angiogenesis, as well as stem and immune cell function [16]. Importantly, while supraphysiological concentrations of TCA intermediates succinate and fumarate can inhibit PHD under normoxia, increased intracellular αKG can reactivate PHD in hypoxic cells resulting in metabolic catastrophe and cell death [17]. Alpha-KG is also required for the activity of some demethylases involved in controlling chromatin modifications and DNA methylation including the ten-eleven translocation (TET) DNA hydroxylases and the Jumonji histone demethylases (Fig. 1). Since histone and DNA methylation have a direct impact on gene transcription, the available pool of αKG modulates cell fate decision. For example, embryonic stem cells exhibit a high level of intracellular αKG to promote histone and DNA demethylation and maintain stem cell self-renewal and pluripotency [18].
Formation of reducing equivalents involved in ATP production, lipid synthesis, and antioxidant defenses. Besides αKG production, the oxidative decarboxylation catalyzed by the 3 IDH isoforms leads to the formation of reducing equivalents, NAD(P)H. IDH3 activity directly generates NADH production as well as FADH2 by promoting TCA cycle running. These reducing equivalents are used by the electron transport chain (ETC) to produce ATP. In contrast, IDH1/2 leads to the formation of NADPH, a key molecule involved in lipid synthesis and the antioxidant machinery (Fig. 1).

Fatty acid and lipid biosynthesis reactions are major users of NADPH. For example, the synthesis of one palmitate (16:0) from acetyl-CoA and malonyl-CoA by fatty acid synthase requires the input of 14 molecules of NADPH. Although the association of NADPH production and lipogenesis is well known, direct evidence of IDH1/2 involvement has been demonstrated only recently. Transgenic mouse overexpressing IDH1 in the liver and adipose tissues experienced obesity and hyperlipidemia, paralleled by increased triglyceride and cholesterol content [19]. Conversely, in vivo IDH1 invalidation resulted in weight loss associated with reduced fat mass and circulating triglycerides levels [20]. In the brain, IDH1 has been shown to regulate phospholipid metabolism in developing astrocytes [21].

Reducing equivalents supplied by NADPH also secure an adequate pool of reduced glutathione (GSH) and thioredoxin to protect the cell from ROS that cause DNA damage, protein oxidation, and lipid peroxidation [22]. The role of IDH1 and IDH2 as protectors against various insults has been confirmed extensively by several groups. Notably, Lee et al. have shown that IDH1 or IDH2 deficiency in mouse embryonic fibroblasts leads to increased lipid peroxidation, oxidative DNA damage, intracellular peroxide generation, and decreased survival after oxidative stress, while overexpression of either IDH1 or IDH2 prevents these effects [23, 24].

Reductive carboxylation as a metabolic adaptation of mitochondrial impairment

The reductive carboxylation is the reverse reaction of the oxidative decarboxylation and can be exclusively catalyzed through IDH1 and IDH2 enzymes using glutamine-derived αKG to produce isocitrate along with NADP+ (Fig. 1). As seen above, pools of reducing equivalents are regulated through an isocitrate/αKG cycle where the irreversible oxidative carboxylation catalyzed by IDH3 is coupled to the reductive decarboxylation catalyzed by IDH2.

Several recent publications revealed the importance of this glutamine-dependent reductive carboxylation for de novo lipogenesis in cells exhibiting mitochondrial dysfunction or upon hypoxia [5, 25, 26]. This reaction allows citrate formation, without passing through the conventional clockwise steps of the TCA cycle, to produce acetyl-CoA and fuel de novo fatty acid biosynthesis, that are key membrane components and important signal transducers. Of note, glutamine-dependent reductive carboxylation has been previously described as a minor source of isocitrate/citrate and lipogenic carbon in a restricted number of normal cells from liver, heart, brown adipocytes, retinal pigment epithelium, and quiescent fibroblasts [27–31]. While αKG/citrate ratio is a critical determinant of glutamine-dependent reductive carboxylation [32], this reaction is inhibited by NADP+ and, to a lesser extent, by isocitrate [33]. Thus, reductive carboxylation retains glutamine as a crucial growth-promoting nutrient when mitochondrial metabolism is impaired.

IDH ENZYMES AS CRUCIAL PLAYERS IN GBM

In 2008, hotspot mutation in IDH1 gene was identified in grade II/III astrocytomas and oligodendrogliomas, and in secondary GBM that developed from these lower-grade lesions [34, 35]. Secondary GBM without IDH1 mutation often had mutations on the IDH2 gene. This was rapidly followed by identification of recurrent IDH1/2 mutations in other tumor types, including acute myeloid leukemia (AML). GBM with IDH mutations are clinically and genetically distinct from GBM with wild-type IDH genes. In particular, patients with mutant IDH1/2 GBM have a better outcome compared to those with wild-type IDH tumor (14 months with wild-type IDH vs 42 months with mutant IDH) (Fig. 2) [36]. Mutant IDH tumors are also associated with extensive epigenomic alterations revealed by a global hypermethylation landscape (G-CIMP phenotype). These particular characteristics prompted the World Health Organization (WHO) in 2021 to refer mutant IDH GBM as grade 4 mutated IDH astrocytoma, to distinguish more clearly between this entity and wild-type IDH GBM [37]. Thus, while IDH enzymes have been known for decades, their contribution to GBM aggressiveness and recurrence has been barely studied until the identification of their mutations. The emerging literature showing how the metabolic functions of IDH enzymes impact tumor initiation, progression, dissemination, and treatment escape in GBM is presented below (Fig. 2).

Metabolic functions of wild-type IDH1 in GBM

Recent studies have highlighted the importance of wild-type IDH1 in GBM progression. First, Calvert et al. reported that wild-type IDH1 is overexpressed in most primary GBM [38]. Notably, in GBM samples profiles provided by The Cancer Genome Atlas Consortium, IDH1 appeared as the most differentially expressed NADPH-producing enzyme compared to normal brain tissue [38, 39] and exhibited a higher maximal enzymatic activity than other NADPH-producing enzymes in patient-derived GBM samples [40]. Through its oxidative decarboxylation activity, IDH1 promotes tumor progression and resistance to cell death through efficient fatty acid synthesis and ROS scavenging activities (Fig. 2) [41]. Accordingly, its genetic or pharmacological inhibition reduced tumor growth, both in vitro and in vivo. Furthermore, and in agreement with its ROS scavenging activity, upregulation of IDH1 expression was observed following ionizing radiation and its silencing increased tumor sensitivity to radiation-induced senescence, both in vitro and in murine xenograft models of human GBM [39]. Finally, rescuing IDH1 metabolic activities was sufficient to reverse this process.

The reductive carboxylation activity of wild-type IDH1 also plays a crucial role in tumor cells located in hypoxic regions, which are frequently found in GBM and have been associated with tumor aggressiveness, invasion, and resistance to therapies. Upon hypoxia, tumor cells rely almost exclusively on glutamine-dependent reductive carboxylation catalyzed by IDH1 for lipids synthesis while, in normoxia, lipids are preferentially synthesized from glucose [5, 25, 42]. Accordingly, knockdown of IDH1 reduced glutamine-dependent reductive carboxylation and impaired cell proliferation, under hypoxia [25].

IDH1-derived αKG can also be transaminated to glutamate through the branched-chain amino acid transaminase-1 (BCAT1). In the brain, glutamate plays a crucial role as a neurotransmitter and also presents clinical relevance in GBM. Indeed, several studies have reported that increased level of glutamate promotes both tumor progression and invasion by providing macromolecule precursors and reducing equivalents for mitochondrial ATP synthesis as well as increasing antioxidant production mainly through GSH synthesis (Fig. 2) [42, 44]. Interestingly, cytoplasmic BCAT1 has been shown to be significantly upregulated in GBM expressing wild-type IDH1 while not being expressed in GBM expressing mutant IDH1 implying a mechanistic link between these two enzymes [45]. This hypothesis was reinforced by the decreased BCAT1 expression in GBM cells upon IDH1 silencing. Importantly, deregulation of either branched-chain amino acid metabolism and glutamate secretion result in neuronal dysfunc-

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Histone & DNA methylation, as well as other epigenetic alterations, are crucial for tumor cell proliferation and survival. The IDH1/2 mutations that occur in GBM lead to the generation of oncometabolites, specifically 2-hydroxyglutarate (2HG), which acts as a competitive inhibitor of α-KG-dependent dioxygenases (α-KGDs) and results in altered DNA and histone methylation patterns. These changes are associated with a more aggressive tumor phenotype, including increased proliferation and decreased differentiation. The mutant IDH1/2 enzymes also alter the metabolism of α-KG, leading to a decrease in the production of NADPH and a concomitant increase in NADH, which affects cell redox status and cellular differentiation. This metabolic shift is accompanied by alterations in cell survival and proliferation under hypoxic conditions, and it contributes to the development of hypoxia and anaplerosis, which are hallmark features of GBM. These metabolic changes also impact histone and DNA methylation, leading to alterations in gene expression and tumor cell behavior. Overall, understanding the metabolic discrepancies between wild-type and mutant IDH1 in GBM is crucial for developing targeted therapies that can reverse these metabolic alterations and improve patient outcomes.
TARGETING OF WILD-TYPE IDH ENZYMES IN GBM

Given the dismal prognosis of GBM, new therapeutic approaches are urgently required. Recent identification of neomorphic IDH1/2 mutations in secondary GBM has generated robust research to elucidate their role in gliomagenesis, tumor progression and impact on clinical outcome. Several small molecules that directly inhibit mutant IDH1/2 activities have been developed, with some of them currently evaluated in phase I/II/III clinical studies in secondary GBM (Table 1) (for detailed review see [68]). However, IDH-targeting therapeutic approaches are currently restricted to mutant IDH1/2 GBM while they represent less than 10% of highly malignant glioma. According to their involvement in a wide range of metabolic processes, wild-type IDH-mediated metabolic reprogramming could also be a key driver of tumor adaptation allowing GBM proliferation, tumor escape, and recurrence. Thus, further investigations should build on mutant IDH1/2 knowledge to propose new therapeutic approaches targeting wild-type isoforms. Here, we will give a brief overview of mutant IDH1/2 inhibitors and discuss wild-type IDH targeting in primary GBM.

Lessons from IDH1/2 mutations in secondary GBM

One striking difference between wild-type IDH GBM and mutant IDH1/2 tumors resides in their methylation landscape, which is known to play important roles during oncogenesis [57] (Fig. 2). Thus, initial preclinical studies used pan-methylases inhibitors such as the FDA-approved drugs 5-azacitidine (5-aza) and decitabine. Treatment of mutant IDH1 GBM bearing mice with either agent resulted in a dramatic loss of stem-like properties and decreased tumor growth [69–71]. However, their impact on the epigenomic landscape of normal cells strongly limits their clinical applications. Furthermore, while DNA hypermethylation elicits tumorigenesis through silencing of tumor suppressor gene, DNA hypomethylation also contributes to oncogenesis through induction of genomic instability and oncogene activation. Since D2HG is sufficient to establish mutant IDH1/2 GBM hypermethylation phenotype, several compounds that directly inhibit mutant IDH1/2 enzymes have been developed with promising results (Table 1). Independently of the targeted mutant isoform, most of them reduced D2HG production in vitro and are able to penetrate the blood–brain barrier. In orthotopic mouse models of mutant IDH1 GBM, their oral administration reduces intratumoral D2HG, reverse histone and DNA hypermethylation, and prolong mice survival [72–74]. Based on these preclinical evidence, several clinical trials are currently ongoing on mutant IDH1/2 glioma, including GBM, to evaluate the safety and efficacy of mutant IDH1 inhibitors (ivosidenib, BAY1436032 and IDH305), mutant IDH2 inhibitor (Enasidenib), and pan-inhibitors inhibiting both mutant isoforms (Vorasidenib) [75–78].

Recently, innovative mutant IDH1 targeting has been published exploiting R132H mutation as a cancer-specific epitope to design protein-specific vaccine [79]. In preclinical syngeneic models, peptide vaccination increased survival of mice bearing mutant IDH1 GBM through CD8+ T cell response, specific cytotoxicity, and an antibody response [80]. A recent phase I trial was carried out in 33 patients with newly diagnosed grade 3 and 4 mutant IDH1 astrocytomas to evaluate the safety and tolerability, as well as immune responses to the peptide vaccine (NOA-16) [81]. NOA-16 demonstrated safety and immunogenicity in 93.3% of patients across multiple MHC alleles. These results are encouraging but the high frequency of pseudoprogression, which was associated with increased vaccine-induced peripheral T cell responses, need further functional investigations using trial tissues.
Table 1. Genetic and pharmacological inhibition of wild-type IDH enzymes.

| Therapeutic agents | Target | Genes | Glioma models | Cellular responses | IC50 IDHmut | IC50 IDHWT | References | Clinical trials (phase) |
|--------------------|--------|-------|---------------|--------------------|--------------|------------|------------|------------------------|
| **Direct chemical inhibitors** |        |       |               |                    |              |            |            |                        |
| Ivosidenib (AGI-120) | IDH1<sup>mut</sup> | Human U87MG cell line | 2-HG production in vivo | 0.25–7 nM | n.d. | [78] | NCT02481154 (I) NCT03343197 (I) NCT04164901 (III) |
| Vorasidenib (AGI-881) | IDH1<sup>mut</sup>/ IDH2<sup>mut</sup> | Human GBM cell lines U87MG and TS603/Murine xenograft of TS603 | ↘ 2-HG production in vivo | 70 nM | 70 nM | [72, 73] |                        |
| Enasidenib (AGI-221) | IDH2<sup>mut</sup> | Human U87MG cell line | ↘ 2-HG production and tumor growth in vivo | 11 nM | 190 nM | [96] | NCT02746081 (I) |
| AGI-5198 | IDH1<sup>mut</sup> | Human U87MG cell line/Murine xenograft of U87MG/Human TS603 GBM cell line/Murine xenograft of TS603 | ↗ histone methylation, ↘ astroglial differentiation, ↘ 2-HG production and tumor growth in vivo | 70 nM | 70 nM | [72, 73] |                        |
| AGI-6780 | IDH2<sup>mut</sup> IDH2<sup>WT</sup> | Human U87MG cell line | ↘ astrocytome proliferation, ↗ cell differentiation ↗ mice survival | 47 nM | 20 µM (cell-free assay) | [97] | NCT02746081 (I) |
| BAY-1436032 | IDH1<sup>mut</sup> IDH1<sup>WT</sup> | Human glioma cell line (LN299)/patient-derived secondary GBM | ↗ tumor proliferation, ↘ cell differentiation ↗ mice survival | 9 nM | 24 µM (cell-free assay) | [98] | NCT03684811 (I-II) 2018-001796-21 (I) |
| FT-2102 | IDH1<sup>mut</sup> | Human U87MG cell line | ↘ tumor proliferation ↗ mice survival, ↗ RTK inhibitor efficacy | n.d. | n.d. | [38] | NCT02381886 (I) |
| IDH-305 | IDH1<sup>mut</sup> | Patient-derived glioma initiating cells/PDXs | ↘ tumor proliferation, ↗ mice survival, ↗ RTK inhibitor efficacy | n.d. | n.d. | [38] | NCT02454634 (I) NCT03893903 (I) |
| GSK264 | IDH1<sup>WT</sup> | Patient-derived glioma initiating cells/PDXs | ↘ tumor proliferation, ↗ mice survival, ↗ RTK inhibitor efficacy | n.d. | n.d. | [38] | NCT02454634 (I) NCT03893903 (I) |
| Immunotherapy |        |       |               |                    |              |            |            |                        |
| Peptide vaccine | IDH1<sup>mut</sup> | Murine GL261 GBM cell line/murine graft of GL261 | ↗ anti-tumor immune response, ↗ mice survival | n.d. | n.d. | [80] | NCT02746081 (I) |
| SirNA/ShRNA therapeutics |        |       |               |                    |              |            |            |                        |
| ShRNA | IDH1<sup>WT</sup> | Patient-derived glioma initiating cells/PDXs | ↘ NADPH and α-KG levels, ↗ cell differentiation and ROS level, ↘ stemness and tumor proliferation, ↗ mice survival | n.d. | n.d. | [38] | NCT02746081 (I) |
| Si/ShRNA | IDH1<sup>WT</sup> | Human GBM cell lines (U87MG, A172 and U138MG)/Murine xenograft of U87MG | ↘ deoxynucleotide and antioxidant pools, ↘ tumor proliferation in combination with irradiation in vivo | n.d. | n.d. | [39] | NCT02746081 (I) |
| SirNA | IDH2<sup>WT</sup> | Human SF188 GBM cell line | ↘ cell proliferation and reductive carboxylation in hypoxia | n.d. | n.d. | [5] | NCT02746081 (I) |
| CRISPR/Cas9 silencing | IDH3<sup>WT</sup> | Patient-derived glioma initiating cells | ↘ NADPH/NAD<sup>+</sup> and nucleotides synthesis, ↘ tumor proliferation ↗ epigenome methylation, methotrexane sensitivity. | n.d. | n.d. | [50] | NCT02746081 (I) |

Several studies have been performed to inhibit wild-type IDH enzymes. Small molecules that directly inhibit mutant IDH1/2 activities have been tested against wild-type IDH enzymes as well as genetic inhibition through RNA interference. n.d. undetermined.
Genetic and pharmacological targeting of wild-type IDHs

In agreement with the crucial role of IDH1 in anti-oxidant defenses through NADPH production, recent studies have demonstrated that its genetic inhibition reduces GBM growth and may significantly improve the efficacy of conventional GBM therapies [38, 39] (Table 1). Indeed, inactivation of IDH1 through RNA interference reduces GBM growth and prolongs the survival of mice bearing patient-derived xenografts. These effects were mediated through inhibition of the oxidative decarboxylation of isocitrate to αKG resulting at the molecular level to impaired lipid and deoxynucleotide biosynthesis and increased ROS production, due to reduced levels of αKG and NADPH. These molecular alterations also resulted in increased tumor cell sensitivity to both radiation-induced senescence and erlotinib-induced apoptosis [38, 39]. Indeed, increased ROS production combined with reduced NADPH and deoxynucleotide pools trigger GSH exhaustion and increase double-strand DNA breaks leading to cell death. One study also reported that wild-type IDH1 silencing significantly reduced the frequency of GBM stem-like cells involved in GBM recurrence [38]. Importantly, they also demonstrated that pharmacological inhibition of wild-type IDH1 recapitulates its genetic silencing [38]. Indeed, GSK864, a compound initially identified as a potent inhibitor of mutant IDH1 in AML [82], inhibits wild-type IDH1 activity, reduces GBM stem-like cell frequency and increases survival of tumor-bearing mice. In contrast to IDH1, no significant metabolic change was observed after IDH2 silencing by RNA interference under normoxia [25]. These results are in agreement with a crucial role of IDH2 in particular conditions such as hypoxia [5].

Genetic inhibition of IDH3α in orthotopic GBM mouse models also decreases cell growth through accumulation of pyrimidine pathway intermediates, increase of total NADPH/NADP⁺ ratio and altered DNA methylation profile [50]. These epigenetic alterations induced by IDH3α deletion deregulate key pathways such as cyclic adenosine 3', 5'-monophosphate-mediated signaling and epithelial-to-mesenchymal transition. Hence, blunted nucleotide biosynthesis, together with epigenetic silencing of potent growth and multipotency factors in response to IDH3α loss of function, creates a unique metabolic vulnerability in highly proliferative GBM cells, that decreases cellular viability. Furthermore, IDH3α extinction cooperates with antifolate therapy, such as methotrexate (MTX), known to target the thymidylate pathway enzymes DHFR and TYMS, to promote programmed cell death [50].

Future strategies to target wild-type IDH enzymes

Published data indicate that mutant as well as wild-type enzymes, are interesting actionable therapeutic targets. Unfortunately, whereas mutant IDH1 inhibitors have been developed, they cannot be directly used in wild-type IDH GBM. First, while some compounds, such as ivosidenib, AGI-6780, and BAY-1436032, may also inhibit wild-type IDH1 activity, required doses are usually too high to be further evaluated in clinics (Table 1). Second, while mutant IDH enzymes display one unique and specific neomorphic activity, wild-type IDH enzymes catalyze several metabolic reactions involved in different cellular processes depending on their intracellular sublocalization and microenvironment. Third, GBM being highly heterogeneous, other factors such as their wider mutational profile, including PS3, PTEN, or EGFR, as well as their molecular signature of their anti-oxidant profiles, may alter wild-type IDH metabolic functions [83]. Finally, a recent computational analysis identified four stable tumor cell states with divergent mitochondrial glucose, glutamine, and lipid metabolism, in addition to specific neurodevelopmental features and different patient outcomes [84]. In particular, the mitochondrial subset of GBM cells relies exclusively on oxidative phosphorylation for energy production, in contrast to glycolytic/plurimetabolic subset sustained by activation of multiple energy-production programs including aerobic glycolysis, amino acids, and lipid metabolism. Thus, the identification of key wild-type IDH-mediated metabolic activity, depending on the genetic and metabolic landscape and involved in GBM aggressiveness, is a prerequisite for further development of specific wild-type IDH inhibitors in preclinical and clinical studies. The canonical function of IDH enzymes, namely the oxidative decarboxylation of isocitrate to αKG, is hardly targetable since it is displayed by most cells, both normal and tumor. However, the reductive carboxylation catalyzed by IDH1 and IDH2 only occurred in anchorage-independent tumor cells, cells with altered mitochondria, or located in hypoxic niches [25, 42, 85]. Accordingly, glutamine-derived reductive carboxylation was barely detected in normoxia and was not affected by IDH1 or IDH2 silencing [25]. In contrast, IDH2-mediated reductive carboxylation becomes critical for tumor proliferation upon hypoxia [5]. This is of particular interest since the most aggressive GBM cells, including GBM stem-like cells and mesenchymal GBM cells, have been shown to reside in hypoxic niches [86–89]. GBM stem-like cells display self-renewal ability and long-term proliferation, present tumor initiation ability, and are radio- and chemo-resistant [90–92]. Mesenchymal GBM cells are predominately present in wild-type IDH GBM, are associated with poor radiation response and worse survival [93]. Importantly, global molecular signatures of most GBM relapses are mesenchymal [94]. Thus, targeting IDH-mediated reductive carboxylation may be a potent way to efficiently eradicate these highly malignant cells while sparing normal cells.

CONCLUSION

In conclusion, wild-type IDH enzymes appear as potent actionable therapeutic target in order to improve primary GBM prognosis. A therapeutic strategy of targeting IDH enzymes via small molecules in combination with targeted and/or conventional therapies could represent a Gordian knot solution and may meet more success than solely targeting genomic alterations in a heterogeneous tumor such as GBM. Importantly, several studies targeting wild-type IDH1 enhances GBM responsiveness to treatments and provides a strong rationale to develop IDH targeted therapies. Finally, since cancers upregulate a variety of metabolic genes that conspire to reprogram tumor cell metabolism to support intense growth and therapy resistance, a deep and investigation of all potential metabolic pathway inhibition in combination, or not with other therapies, should hopefully lead to therapeutic advances that will improve the dismal outcomes currently seen for GBM patients.

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