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Metabolic consequences of oncogenic IDH mutations

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ABSTRACT

Specific point mutations in isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) occur in a variety of cancers, including acute myeloid leukemia (AML), low-grade gliomas, and chondrosarcomas. These mutations inactivate wild-type enzymatic activity and convey neomorphic function to produce D-2-hydroxyglutarate (D-2HG), which accumulates at millimolar levels within tumors. D-2HG can impact α-ketoglutarate-dependent dioxygenase activity and subsequently affect various cellular functions in these cancers. Inhibitors of the neomorphic activity of mutant IDH1 and IDH2 are currently in Phase I/II clinical trials for both solid and blood tumors. As IDH1 and IDH2 represent key enzymes within the tricarboxylic acid (TCA) cycle, mutations have significant impact on intermediary metabolism. The loss of some wild-type metabolic activity is an important, potentially deleterious and therapeutically exploitable consequence of oncogenic IDH mutations and requires continued investigation in the future. Here we review how IDH1 and IDH2 mutations influence cellular metabolism, epigenetics, and other biochemical functions, discussing these changes in the context of current efforts to therapeutically target cancers bearing these mutations.

Contents

1. Introduction

Mutations in oncogenes and tumor suppressors facilitate the rapid growth of cancer cells and their survival in response to environmental

stress. To maintain this phenotype tumor cells initiate a metabolic program that supplies the energy, reducing equivalents, and biosynthetic precursors necessary to divide (Tennant et al., 2010). In the early 20th century Otto Warburg observed that cancer cells (and normal proliferating cells) selectively metabolized glucose to lactate even under aerobic conditions (Warburg, 1956). This phenomenon, also known as the "Warburg Effect", is common to many (but not all) tumors. Since Warburg's discovery, biochemists have painstakingly annotated the network of biochemical reactions comprising cellular metabolism. Though not yet complete, this information provides a biochemical roadmap to study metabolic dysfunction in the context of diseases using a range of datasets (Yizhak et al., 2010; Bordbar et al., 2014). Most oncogenes and tumor suppressors directly impact cellular metabolism,

and several hallmark cancer mutations have been observed to occur in metabolic enzymes. Homozygous loss-of-function mutations in fumarate hydratase (FH) or one of the five subunits comprising the succinate dehydrogenase (SDH) complex can lead to the development of specific cancers, representing the first time that metabolic enzymes were classified as bonafide tumor suppressors (King et al., 2006). More recently, mutations in isocitrate dehydrogenase 1 and 2 (IDH1, IDH2) have been discovered in various cancers. These exclusively heterozygous mutations do not follow a traditional loss-of-function mechanism, and the downstream effects of these mutations on tumor initiation, metabolism, and growth are currently being investigated. Here we review how mutations in IDH1 and IDH2 impact intermediary metabolism and other cell functions. Finally, the metabolic and epigenetic consequences of mutant IDH1 and IDH2 are discussed in the context of current efforts to therapeutically target cancers harboring these mutations.

2. Mutation of isocitrate dehydrogenase 1 and 2

Mutations of *IDH1* and *IDH2* were initially identified through exome sequencing of colon tumor and glioblastoma multiforme (GBM) (Sjoblom et al., 2006; Parsons et al., 2008). Since these discoveries, IDH mutations have been observed in several other tumor types, including acute myeloid leukemia (AML), chondrosarcoma, and intrahepatic cholangiocarcinoma (Parsons et al., 2008; Mardis et al., 2009; Yan et al., 2009; Amary et al., 2011; Borger et al., 2012). These mutations are somatically acquired and occur on distinct arginine residues of IDH1 (R132) and IDH2 (R172 or R140). Interestingly, *IDH1* mutations occur at much higher incidences than *IDH2* mutations in low grade gliomas, cholangiocarcinoma, and chondrosarcoma; however, *IDH1* and *IDH2* mutations occur at similar rates in AML (Ward et al., 2010; Molenaar et al., 2014). Due to the frequency of observation in low grade gliomas, IDH mutations are thought to play a significant role in early tumorigenesis and precede other oncogenic mutations (Balss et al., 2008; Watanabe et al., 2009; Juratli et al., 2013).

In contrast to SDH and FH mutants, which exhibit traditional homozygous loss-of-function mutations, IDH mutants retain one wild-type allele and rarely exhibit loss of heterozygosity (Mullen & DeBerardinis, 2012; Jin et al., 2013). Furthermore, the occurrence of mutations on distinct IDH1 and IDH2 residues within the active site provided evidence that these changes elicit a gain-of-function phenotype in each enzyme. Subsequently, an analysis of the x-ray structure of mutant IDH1 in conjunction with metabolomics profiling demonstrated that (D)-2-hydroxyglutarate (D- or R-2HG) was produced by mutant IDH1 and

accumulated at high levels in mutant tumors, confirming a gain-of-function mechanism (Dang et al., 2009). Similar production of D-2HG was demonstrated in cells and tumors harboring *IDH2* mutations (Ward et al., 2010).

Wild-type IDH1 and IDH2 normally catalyze the reversible, NADP + -dependent oxidative decarboxylation of isocitrate to alpha-ketoglutarate in either the cytosol (IDH1) or mitochondria (IDH2). However, the mutant IDH enzyme loses oxidative activity and instead reduces alpha-ketoglutarate (αKG, also known as 2-oxoglutarate) to D-2HG, consuming one molecule of NADPH in the process (Fig. 1). Under normal conditions human cells produce low levels of both D-2HG and L-2HG (or S-2HG) due to enzyme promiscuity, but 2HG (referring to both D-2HG and L-2HG) fails to accumulate due to the activity enantiomer-specific FAD-dependent 2-hydroxyglutarate dehydrogenases (*L2HGDH* and *D2HGDH*) that convert 2HG to αKG (Van Schaftingen et al., 2013). Deficiency in *L2HGDH* or *D2HGDH* due to homozygous loss-of-function mutation causes patients to develop 2HG aciduria characterized by an accumulation of either enantiomer in body fluids (Struys, 2006; Rzem et al., 2007). About 50% of patients with D-2HG aciduria have autosomal recessive mutations in *D2HGDH*; however, the majority of patients with normal D-2HGDH enzyme but high D-2HG harbored mutations in *IDH2* (either R140Q or R140G) (Kranendijk et al., 2010). Patients with D-2HG aciduria either show no symptoms or exhibit developmental delay, epilepsy, cardiomyopathy, and other clinical symptoms (Kranendijk et al., 2010). In contrast, patients with L-2HG aciduria have an increased risk of developing certain brain cancers, suggesting that 2HG may act as a driver of tumorigenesis (Moroni et al., 2004; DeBerardinis & Thompson, 2012). Most patients who developed metastatic brain tumors exhibited high levels of L-2HG, not D-2HG, and tumors that develop are of a different type than those commonly associated with IDH mutation (Cairns & Mak, 2013). Thus, D-2HG accumulation from mutant IDH may not be sufficient to drive malignancy and may require additional oncogenic mutations. Indeed, IDH mutations observed in low-grade gliomas frequently precede 1p/19q co-deletion and/or *TP53* mutation which give rise to either oligoastrocytomas/oligodendrogliomas or low grade astrocytomas, respectively (Labussiere et al., 2010; Lai et al., 2011; Ichimura, 2012; Cairns & Mak, 2013). These tumors follow distinct transformation programs with 1p/19q co-deleted tumors commonly activating PI3K/Akt or Ras and p53 mutant tumors amplifying receptor tyrosine kinases (i.e., MET and PDGFR) (Wakimoto et al., 2014). Further transformation of IDH mutant low-grade gliomas into secondary glioblastomas requires EGFR amplification, PTEN loss, and/or additional genetic alterations (Lai et al., 2011).
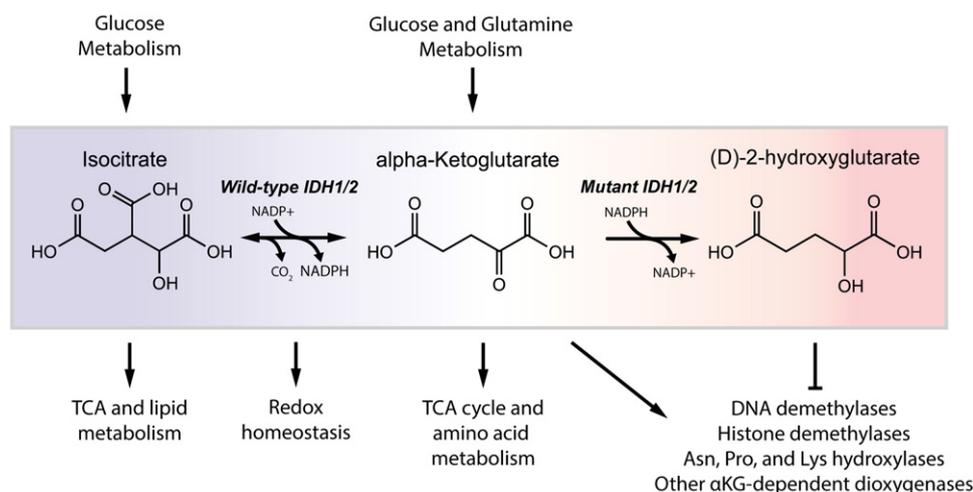

Fig. 1. Multiple cellular pathways are affected by mutations in *IDH1* and *IDH2*. Metabolites involved in these reactions are critical for glucose, glutamine, NADPH, amino acid, and lipid metabolism as well as epigenetic regulation.

Sequencing of *IDH1* and *IDH2* in AML patients indicated that these mutations occurred in a subset of tumors that were distinct from those harboring loss-of-function *TET2* mutations, suggesting that D-2HG accumulation disrupts the function of TET2 or another αKG-dependent dioxygenase (Figueroa et al., 2010). Several studies have subsequently indicated that both L-2HG and D-2HG can act as competitive inhibitors of αKG-dependent dioxygenases, including the EglN family of prolyl hydroxylases (PHDs), the TET family of DNA demethylases, and the JmjC family of histone demethylases (Chowdhury et al., 2011; Xu et al., 2011; Koivunen et al., 2012; Lu et al., 2012). As such, D-2HG acts in a manner similar to the succinate and fumarate that accumulate in the context of SDH and FH mutant tumors, respectively (Selak et al., 2005; Xiao et al., 2012). Indeed, D-2HG accumulation resulting from mutant IDH expression has been observed to promote DNA and/or histone hypermethylation phenotypes (Figueroa et al., 2010; Lu et al., 2013; Turcan et al., 2012). At least in the context of SDH mutant cells, this inhibitory effect on dioxygenase activity can be ameliorated by addition of cell-permeable αKG analogs (MacKenzie et al., 2007). In addition, histone hypermethylation associated with IDH1 mutant expression in U87 glioma cells was reversed by octyl-αKG addition (Xu et al., 2011). In contrast to the inhibitory mechanisms noted above, D-2HG has been observed to activate EglN in many cell and in vivo models, leading to hypoxia inducible factor-1α (HIF1α) degradation and the promotion of tumor development (Koivunen et al., 2012; Losman et al., 2013). On the other hand, other studies have observed increases in HIF1α levels in IDH1 mutant U87 cells (Zhao et al., 2009; Xu et al., 2011) or in brain-specific Nestin-IDH1$^{R132H/wt}$ transgenic mouse embryos (Sasaki et al., 2012a). In contrast, an analysis of IDH1-R132H and HIF1α expression in serial sections of IDH1-R132H positive gliomas suggested that IDH1-R132H expression was not sufficient for HIF1α stabilization (Williams et al., 2011). Overall, the role of IDH mutants and 2HG on HIF1α stabilization is complex and can be influenced by cell type, tissue, and the local microenvironment. The epigenetic dysregulation caused by αKG antagonism has been proposed to be one mechanism through which D-2HG contributes to tumorigenesis in mutant IDH tumors (Fig. 1). However, the specific αKG-dependent dioxygenases that contribute to tumor development are likely to be context-dependent (e.g. tissue specific). As this family of enzymes catalyzes a wide variety of reactions and includes protein- and DNA-modifying enzymes as well as metabolic enzymes (reviewed by Losman & Kaelin, 2013), additional insights are needed to determine the mechanistic drivers of tumorigenesis downstream of mutant IDH (Losman & Kaelin, 2013).

3. Isocitrate dehydrogenase mechanism and regulation

The crystal structures of human IDH1 and pig IDH2, which shares >97% identity with human IDH2, have yielded insights into the enzymatic and regulatory mechanisms of these NADP + -dependent enzymes (Ceccarelli et al., 2002; Xu et al., 2004). Structural studies of IDH1 suggest that its IDH1 follows a self-regulation feedback mechanism whereby isocitrate binds directly to Arg132, inducing a conformational change that allows the Asp279 residue to interact with Ca2+ cofactor and participate in catalysis (Xu et al., 2004). Kinetic studies suggest that isocitrate, and to a greater extent NADP+, regulate the activity and directionality of IDH1 (Rendina et al., 2013). The point mutations in IDH1 and IDH2 have significant effects on enzyme catalytic function and mechanism (Dang et al., 2009; Rendina et al., 2013). Arg132 directly interacts with isocitrate, and amino acid substitutions from any of the mutations observed in gliomas prevented isocitrate from binding (Dang et al., 2009; Zhao et al., 2009). Thus, IDH1 mutants become insensitive to physiological isocitrate levels and exhibit a >80% decreased capacity to carry out the oxidative reaction (Zhao et al., 2009). Consequently, the NADPH production by oxidative IDH activity is diminished, resulting in a ~38% reduction in the NADPH

generation capacity in *IDH1*-mutant versus wild-type glioblastoma tumor tissue (Bleeker et al., 2010).

IDH1 mutants exhibit a sequential kinetic mechanism whereby NADPH first binds, reductively trapping αKG into D-2HG before allowing it to undergo carboxylation to form ICT (Rendina et al., 2013). IDH1-R132 variants (H, C, G, S, L) exhibit significantly different kinetic parameters for αKG and, consequently, produce different levels of D-2HG in cells expressing IDH1-R132 (Pusch et al., 2014). These mechanistic insights offer an explanation as to why D-2HG is preferentially produced by mutant IDH1 enzymes. In addition to the IDH1-R132 and IDH2-R172 and R140Q mutants, other IDH mutation sites have been predicted and/or demonstrated to exhibit neomorphic activity, including IDH1-R100, IDH1-Y179, and IDH1-G97 (Ward et al., 2012; Rendina et al., 2013). IDH1-Y179 and IDH1-G97 mutants exhibited lower Km values for isocitrate (i.e., improved binding); suggesting that neomorphic function is not reliant on an impaired utilization of isocitrate (Rendina et al., 2013). Ultimately, the changes in wild-type and neomorphic function of IDH1 and IDH2 described above influence cell signaling, epigenetics, and enzyme activity to directly and indirectly drive metabolic reprogramming within tumors (Fig. 2).

4. Glucose metabolism

Glycolytic flux is commonly upregulated in tumors downstream of various signaling pathways. For example, phosphoinositide 3-kinase (PI3K) is activated in many tumors and plays a significant role in maintaining the glycolytic phenotype of cancers through protein kinase B (PKB/Akt) signaling (Engelman, 2009). This oncogenic signal stimulates glycolysis, in part, by promoting the expression of glucose and other nutrient transporters and stimulating the activity of glycolytic enzymes including hexokinase and PFKFB3 (Elstrom et al., 2004; DeBerardinis et al., 2008; Vander Heiden et al., 2009; Cairns et al., 2011). While hyperactivation of PI3K/Akt signaling contributes to the aggressiveness of gliomas (Bleau et al., 2009; Koul, 2008), U87 glioma cells expressing IDH1-R132H exhibited decreased Akt levels at both the mRNA and protein level (Bralten et al., 2011). Furthermore, expression of mutant IDH1 in LN-319 glioblastoma cells caused a decrease in Akt phosphorylation, suggesting that mutant tumors may exhibit less of a glycolytic metabolic phenotype compared to IDH1 wild-type tumors (Birner et al., 2014). Importantly, the majority of tumors harboring both IDH mutations and 1p/19q co-deletion exhibit activation of PI3K/Akt; thus, the role of Akt on glucose metabolism in mutant IDH tumors may also rely on external factors (i.e. additional oncogenic mutations, tumor microenvironment).

Lactate dehydrogenase A (*LDHA*) is commonly upregulated in cancer cells downstream of HIF1α and Myc signaling (Kim et al., 2007; Dang et al., 2008; Cairns et al., 2011); this enzyme helps maintain glycolysis via NAD+ regeneration (Metallo & Vander Heiden, 2013). One recent study observed that IDH mutant gliomas and tumor-derived brain tumor stem cells silence *LDHA* expression through promoter hypermethylation (Chesnelong et al., 2014). In addition, one study identified that AML patients with *IDH1 or IDH2* mutant tumors exhibited lower LDH activity compared to wild-type *IDH1* or *IDH2* tumors, suggesting a common phenotype between *IDH* mutants mediated by αKG/2HG (Chou et al., 2011). An analysis of gene expression in *IDH1* mutant and *IDH1* wild-type glioma samples demonstrated that factor inhibiting HIF-1 (FIH-1/HIFAN) was upregulated in *IDH1* mutant tumors (Mustafa et al., 2014). FIH-1 inhibits the activity of HIF1α by preventing its transactivation in an αKG-dependent manner (Mahon et al., 2001), and HIF1α levels are known to be decreased in IDH1 mutant gliomas, enhancing tumorigenesis (Koivunen et al., 2012). This phenotype is thought to result from increased EglN activity fueled by 2HG-αKG isomerization (Tarhonskaya et al., 2014). Putatively as a consequence of HIF1α suppression, IDH1 mutant gliomas expressed high levels of *LDHB* relative to *LDHA* as compared to IDH1 wild-type gliomas or normal brain tissue (Mustafa et al., 2014). Acting as the final step in converting
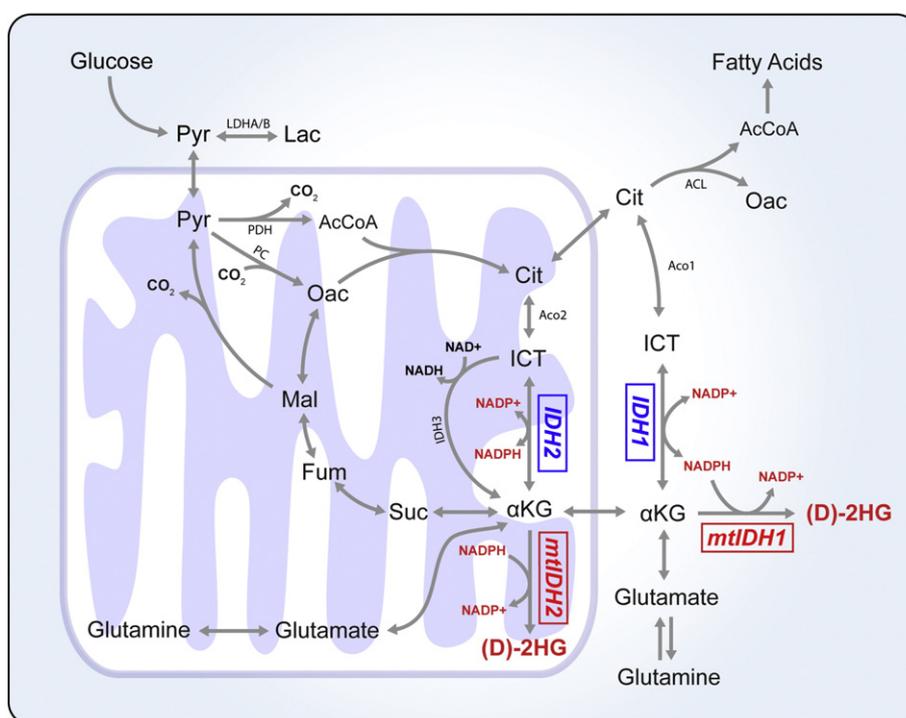
Fig. 2. Biochemical pathways involved in intermediary metabolism. Glycolysis and glucose entry into the TCA cycle is regulated by the activity of lactate dehydrogenase (LDHA and LDHB), pyruvate dehydrogenase (PDH), and pyruvate carboxylation (PC). IDH1 and IDH2 are cytosolic and mitochondrial enzymes, respectively, that are critical for the metabolism of glucose- and glutamine- derived carbons. NADPH produced by either IDH1 or IDH2 is critical for maintaining the redox state in subcellular compartments. AcCoA: acetyl-coenzyme A, αKG: alpha-ketoglutarate, Cit: citrate, D-2HG: D-2-hydroxyglutarate, Fum: fumarate, ICT: isocitrate, Lac: lactate, Mal: malate, Oac: oxaloacetate, Pyr: pyruvate, Suc: succinate.

glucose to lactate, *LDHA* silencing may act to mitigate aerobic glycolysis. Although both isoforms of LDH (LDHA and LDHB) can metabolize the conversion of pyruvate to lactate, the LDHB isoform is more sensitive to substrate inhibition by pyruvate and is more capable of converting lactate to pyruvate (Dang, 2013).

Pyruvate dehydrogenase (PDH) is a major point of entry for glucose-derived pyruvate oxidation in the TCA cycle. PDH activity is regulated by its phosphorylation state and HIF1α stimulates expression of PDK1, leading to inactivating phosphorylation of PDH (Semenza et al., 1994; Kim et al., 2006; Papandreou et al., 2006; Rardin et al., 2009). Thus, alteration of HIF1α expression in mutant IDH cells and tumors may influence PDH activity and lead to changes in flux of glucose-derived pyruvate into the mitochondria. In addition to mitochondrial acetyl-CoA generated by PDH, cells need oxaloacetate (OAC) to maintain TCA cycle flux. Cancer cells can obtain OAC through various mechanisms, including glucose anaplerosis via pyruvate carboxylase or glutaminolysis, and pyruvate carboxylase is required for cells growing in glutamine-deprived conditions (Cheng et al., 2011). Furthermore, in vivo tracing studies in an orthotopic model of human glioblastoma using 13C-labeled glucose have indicated that pyruvate carboxylase and PDH are highly active in GBM (Marin-Valencia et al., 2012). One recent study demonstrated that IDH mutant overexpression in astrocytes results in an increased fractional flux through pyruvate carboxylase and an increase in PC expression, suggesting this pathway is critical for IDH mutant cells to maintain TCA activity (Izquierdo-Garcia et al., 2014). Consistent with this observation, we observed increased pyruvate cycling through malic enzymes and PC in HCT116 cells harboring heterozygous *IDH1* mutations (Grassian et al., 2014).

5. Glutaminolysis, reductive carboxylation, and tricarboxylic acid metabolism

Glutamine is another major contributor to TCA metabolism in cancer cells and enters this pathway at αKG. As such, glutamine–glutamate-αKG metabolism represents a critical node in IDH mutant tumors.

Glutamine is converted to glutamate during the biosynthesis of nucleotides, hexosamines, and asparagine; alternatively this reaction may be catalyzed in mitochondria via glutaminase (GLS). Transaminases or glutamate dehydrogenase (GLUD) can convert glutamate to the TCA intermediate and IDH substrate/product αKG. These pathways are highly active in most cancer cells as a result of oncogenic mutations or limited glucose oxidation (Gaglio et al., 2011; Son et al., 2013). Hypoxic micro-environments common to solid tumors promote glutamine flux into TCA metabolism such that it becomes the predominant carbon source for the glutaminolysis (Le et al., 2012; Fan et al., 2013; Grassian et al., 2014) and reductive carboxylation pathways (Scott et al., 2011; Wise et al., 2011; Metallo et al., 2012; Mullen et al., 2012).

Not surprisingly, much of the D-2HG produced by mutant IDH1 and IDH2 in cells is derived from glutamine (Grassian et al., 2014). Due to its clinical prevalence and the availability of cell models more studies have focused on the impact of mutant IDH1 on TCA metabolism compared to mutant IDH2. Beyond αKG generation, flux through both glutaminolytic and reductive carboxylation pathways are significantly impacted by IDH mutations. When oxygen is replete, evidence suggests that IDH flux predominantly occurs in the oxidative direction, with minimal (but some) exchange observable. Given the impact of mutant IDH1 on WT activity it is not surprising that such cells become more reliant on the glutaminolysis pathway. Recent studies have highlighted differences in this pathway when cells express or harbor *IDH1* mutations. For example, a glioblastoma cell line and transformed astrocytes both exhibited increased sensitivity to pharmacological or siRNA-mediated inhibition of glutaminase (Seltzer et al., 2010). In addition, Chen et al. recently observed that gliomas harboring *IDH1* mutations overexpressed glutamate dehydrogenase 1 and 2 (*GLUD1* and *GLUD2*), and orthotopic growth of mutant glioma lines were sensitive to *GLUD1* or *GLUD2* knockdown (Chen et al., 2014). We observed a similar increase in the dependence of IDH1 mutant cells on glutaminolysis in our analysis of a panel of HCT116 cells, providing evidence that these changes arise due to a direct impact on metabolism rather than indirectly through cell lineage-specific mechanisms (Grassian et al., 2014). Notably, this

dependence on oxidative glutamine metabolism was exacerbated by culture under hypoxia, such that mutant IDH1 cells exhibited decreased growth and increased respiration under hypoxia (Grassian et al., 2014).

IDH1 has been implicated in catalyzing the reductive carboxylation of αKG to isocitrate, a pathway that facilitates conversion of glutamine to biosynthetic intermediates under conditions of hypoxia or mitochondrial dysfunction (Scott et al., 2011; Metallo et al., 2012; Mullen et al., 2012). As the IDH reactions in human cells involve the interplay of NADH, NADPH, αKG, and isocitrate in two important cellular compartments, the localization, interconnectivity (i.e., via NAD(P)H shuttling), regulation, and function of the reductive carboxylation pathway is still actively investigated. In vitro enzyme studies have demonstrated that mutant/wild-type heterodimers of both IDH1 and IDH2 are unable to catalyze the reductive carboxylation reaction (Leonardi et al., 2012). Given the demonstrated role of IDH1 in this reaction, mutant IDH1 cells exhibit a strong defect in the conversion of glutamine to isocitrate, citrate, and acetyl-CoA under various conditions. Indeed, the extent that heterozygous mutant IDH HCT116 cells and IDH1-R132C HT1080 fibrosarcoma cells activate this pathway under hypoxia was compromised when compared to cells with IDH2 mutations or wild-type IDH (Grassian et al., 2014). Changes in glutamine metabolism under hypoxia were also observed in an additional study that employed HCT116 IDH1-R132H cells (Reitman et al., 2014). Furthermore, IDH1 mutant cells exhibited increased sensitivity to inhibitors of respiration, conditions known to promote reductive carboxylation (Mullen et al., 2012; Fendt et al., 2013; Gameiro et al., 2013). This sensitivity could be due to the cells' inability to synthesize acetyl-CoA through reductive carboxylation or alternatively due to their increased dependence on respiration under hypoxia. TCA metabolism is coupled with cellular respiration. As noted above, we observed increased sensitivity to ETC/respiration inhibitors and changes in oxygen consumption rates in IDH1 mutant HCT116 cells under hypoxia (Grassian et al., 2014). More recently, Chan et al. and other studies have demonstrated that D-2HG produced by mutant IDH inhibits complex IV (also known as cytochrome c oxidase, COX) of the ETC (da Silva et al., 2002; Wajne et al., 2002; Latini et al., 2005; Chan et al., 2015). This mechanism induced mutant IDH leukemia cell lines (patient-derived and engineered) to become sensitive to Bcl-2 inhibitors, initially identified as a target in a shRNA screen (Chan et al., 2015).

In addition to fatty acid and cholesterol synthesis, acetyl-CoA is an important building block for phospholipids, amino acids, and protein acetylation (Kaelin & McKnight, 2013). Interestingly, N-acetylated amino acids including N-acetyl-aspartyl-glutamate (NAAG) and N-acetyl-aspartate (NAA) were significantly decreased in human glioma cells expressing IDH1-R132H (Reitman et al., 2011). These results suggest that mutant IDH tumors may exhibit perturbed acetyl-CoA metabolism, potentially due to changes in pathway fluxes fueling acetyl-CoA pools. In addition to differences in acetyl-CoA metabolism, IDH mutant tumors exhibited a significantly altered phospholipid profile compared to wild-type IDH tumors. Specifically, pools of the phospholipid metabolites phosphoethanolamine and glycerophosphocholine were significantly perturbed in mutant IDH versus wild-type IDH tumors (Esmaeili et al., 2014). Several of the oncogenic signaling pathways altered in IDH mutant tumors also impact fatty acid synthesis and uptake. For example, ATP-citrate lyase (ACL) acts as the major supplier of cytosolic AcCoA for fatty acid synthesis and is a major Akt substrate, and Akt also induces other fatty acid synthesis enzymes (i.e. FAS, ACC) via mTORC1 activation of SREBP-1 (Berwick et al., 2002; Porstmann et al., 2008; Ru et al., 2013). In addition to de novo synthesis, fatty acids and cholesterol can be scavenged from extracellular sources, and PI3K/Akt signaling can upregulate expression of the LDL receptor—supplying cells with cholesterol—via SREBP-1 (Guo et al., 2011). Of note, IDH1 is a transcriptional target of SREBP-1a and to a lesser extent SREBP-2, purportedly to supply NADPH for reductive biosynthesis in the cytosol (Shechter et al., 2003).

In part due to a lack of isogenic or cell-based models, fewer studies have addressed the impact of heterozygous mutations in IDH2 on intermediary metabolism. Generally, cells expressing IDH2-R172 accumulate more or similar amounts of D-2HG than those with mutant IDH1, though IDH2-R140Q mutants produce the least (Ward et al., 2010). In vitro enzyme studies and ectopic expression of mutants has indicated that differences in gene expression and compartment localization/conditions may influence the differential D-2HG production by IDH1-R132 mutants versus IDH2-R172 mutants (Ward et al., 2013). In addition to effects on D-2HG accumulation, some evidence indicates that the intermediary metabolism of cells with mutant IDH1 versus IDH2 differs as well. As noted above, detectable and significant effects on TCA metabolism were observed in an isogenic HCT116 cell panel cultured under normoxia and hypoxia. In contrast, the profile of glucose and glutamine-driven TCA metabolism in cells with either IDH2-R172 or IDH2-R140Q mutations was similar to that of parental cells cultured normally or in the presence of exogenous D-2HG (Grassian et al., 2014). No in vitro growth defect was observed under hypoxia, and the cells readily used the reductive carboxylation pathway for de novo lipogenesis. Similar trends were observed when comparing HT1080 (IDH1-R132C) fibrosarcoma and SW1353 (IDH2-R172S) chondrosarcoma cells.

In the context of analyzing flux changes in heterozygous IDH mutant tumors versus those with WT IDH1 and IDH2 it is important to consider that a heterozygous mixture of homo- and heterodimers will exist within cells. Notably, the binding affinity of IDH1-R132 and IDH1-WT monomers was not significantly different, suggesting that a diversity of homo- and hetero- IDH1 dimers exists in *IDH1* mutant tumors (Jin et al., 2011). In contrast, IDH2-R172 weakly binds IDH2-WT, indicating that there is a greater enrichment of WT–WT homodimers in mutant IDH2 tumors (Jin et al., 2011). Furthermore, differences in substrate availability for the IDH reaction are likely significant when comparing metabolism in the cytosol/peroxisome (IDH1) and mitochondrial matrix (IDH2). The ability to resolve such differences remains a challenge, as it is unclear to what extent reductive carboxylation is catalyzed in the mitochondria versus cytosol (Metallo & Vander Heiden, 2013). While defects in this pathway arise in mutant IDH1 cells but not mutant IDH2 cells, the expression of several mitochondrial enzymes (e.g. transhydrogenase, αKG-dehydrogenase complex) influences reductive carboxylation flux (Mullen et al., 2012; Gameiro et al., 2013). Despite the observed in vitro differences in metabolism and growth, HCT116 cells containing either *IDH1* or *IDH2* mutations panel grew significantly slower as xenografts when compared to parental cells (Grassian et al., 2014). These findings highlight the importance of microenvironment on metabolism and the impact of IDH mutations as well as the need for better cell/tumor models.

6. Other metabolic pathways

Beyond glucose, glutamine, and acetyl-CoA metabolism, αKG and 2HG can influence a number of other metabolic pathways. As noted above, D-2HG may inhibit (or promote the activity of) other members of the αKG-dependent dioxygenase family. These enzymes catalyze diverse functions that include various metabolic reactions beyond demethylation (Rose et al., 2011; Losman & Kaelin, 2013). For example, the activity of several proline and lysine hydroxylases are perturbed in the context of IDH mutations, leading to compromised collagen maturation and impacts on extracellular matrix (ECM) processing. Notably, a significant impact on ECM was observed in an IDH mutant knock-in model (Sasaki et al., 2012b). The dioxygenase family also includes enzymes involved in fatty acid metabolism, RNA modifications, and carnitine biosynthesis (Rose et al., 2011; Losman & Kaelin, 2013). Furthermore, αKG is a substrate for a large number of enzymes outside of dioxygenases, including transaminases. Notably, BCAT1 expression is high in glioblastoma and suppressed by ectopic mutant IDH1 overexpression, suggesting that αKG or D-2HG levels influence branched

chain amino acid (BCAA) metabolism (Tonjes et al., 2013). The activities of aspartate aminotransaminase (AST) and glutamate dehydrogenase (GDH), enzymes that utilize aKG as a substrate, were decreased in IDH1 mutant U87 cells due to changes in expression (AST) or posttranslational modifications (GDH) (Chaumeil et al., 2014). More focused, functional characterization of these pathways may highlight additional metabolic perturbations in IDH mutant tumors.

7. Redox metabolism

In addition to the aforementioned TCA intermediates, the reactions catalyzed by IDH1 and IDH2 require NADPH as a cofactor (Fig. 2). The pyridine nucleotide cofactor NADP(H) is critical for important cellular processes supporting redox homeostasis and biosynthesis of lipids and nucleotides (Pollak et al., 2007). NADP(H) exists as either the oxidized (NADP+) or reduced (NADPH) form, and the ratio of this redox couple heavily influences cellular physiology. The regeneration rate of reduced NADPH is extraordinarily high in proliferating cancer cells such that the pool turns over in approximately 20 min (Fan et al., 2014; Lewis et al., 2014). Classically, NADPH was thought to be regenerated primarily by the oxidative pentose phosphate pathway (PPP); however, recent evidence suggests that several other enzymes are also major contributors (Pollak et al., 2007; Fan et al., 2014; Lewis et al., 2014). These enzymes include malic enzymes (ME), isocitrate dehydrogenases (IDH), aldehyde dehydrogenases (ALDH), and methylene tetrahydrofolate dehydrogenases (MTHFD) (Pollak et al., 2007; Tibbetts & Appling, 2010; Lunt & Vander Heiden, 2011). Importantly, many of these enzymes have isoforms that exist in specific organelles (e.g., ME1 is cytosolic while ME2 and ME3 are mitochondrial), and since NADP(H) cannot transport directly across subcellular organelle membranes the maintenance of redox homeostasis in each organelle is distinctly regulated.

Mutation in either IDH1 or IDH2 deactivates the NADPH-production capacity of these enzymes; thus, mutant IDH cells may need to reroute flux through compensatory NADP + -dependent enzymes or suffer a decrease in available NADPH. In order to prevent oxidative damage from reactive oxygen species (ROS) generated during proliferation cells must maintain pools of reduced glutathione (GSH), the most abundant cellular antioxidant (Balendiran et al., 2004). Reduced glutathione can either be synthesized de novo or regenerated from oxidized glutathione (GSSG) via NADPH and glutathione reductase. A reduction in NADPH availability could lead to an increase in oxidative stress by decreasing GSH pools. In fact, one study of clonally selected cells overexpressing wild-type or IDH1-R132H glioma cells indicated that NADPH levels were decreased relative to wild-type IDH1 cells (Shi et al., 2014). Consequently, ROS and GSH levels were increased and decreased, respectively, in cells expressing mutant IDH1 (Shi et al., 2014). Furthermore, mutant IDH1 cells exhibited increased sensitivity to temozolomide (TMZ) and cis-diamminedichloroplatinum (CDDP), which can induce oxidative stress in tumor cells (SongTao et al., 2012; Shi et al., 2014). A similar sensitivity was observed when cells ectopically expressing mutant IDH1 or IDH2 were exposed to radiation (Li et al., 2013). These data provide some indication that oncogenic IDH1 perturbs NADPH homeostasis; however, the extent that these findings correlate with survival and treatment responsiveness remains unclear (Dubbink et al., 2009; Houillier et al., 2010). Ultimately, additional molecular studies are required to elucidate whether this increased sensitivity to oxidative stress is due to an inability to compensate metabolically or because of orthogonal effects of the mutation on cell physiology/ epigenetics.

An in vivo knock-in model of IDH1-R132H exhibited increased NADP+/NADPH ratio, decreased GSH, and decreased ascorbate in whole brains, consistent with a decrease in NADPH production and redox control capacity (Sasaki et al., 2012a). However, intracellular ROS levels in total brains of IDH1-R132H knock-in mice were significantly reduced relative to IDH1-WT knock-in brains (Sasaki et al., 2012a). Inhibition of IDH1-R132H may increase total ROS levels and,

along with reduced NADPH and GSH levels, increase oxidative stress in these tumors and lead to cell death. High levels of ROS can damage lipids, proteins, and DNA and can lead to the activation of apoptosis and disruption of the cell cycle (Finkel & Holbrook, 2000). In addition, high levels of mitochondrial ROS may contribute significantly to mitochondrial dysfunction, as mtDNA is more readily damaged than nuclear DNA (Kim et al., 2015). Mutation of mtDNA has been observed to contribute to tumorigenicity in several cancer types (Sabharwal & Schumacker, 2014); to this end, mutant IDH2 cells in particular may exhibit compromised mitochondrial NADPH homeostasis, which may lead to increased mtDNA mutation and mitochondrial dysfunction.

8. Allelic inhibitors of mutant isocitrate dehydrogenase 1 and 2

Given the distinct, gain-of-function activity caused by IDH mutations, several efforts have identified selective pharmacological agents that target mutant IDH1 and IDH2 enzymes. One of the first compounds (AGI-5198) to be discovered specifically inhibited IDH1-R132H and IDH1-R132C mutant enzymes, reduced 2HG levels in glioma cells, and impaired growth of IDH1 mutant but not IDH1 WT glioma xenografts (Rohle et al., 2013). AGI-5198 suppression of 2HG levels did not completely ameliorate the DNA hypermethylation phenotype in mutant IDH1 glioma cells, suggesting that mutant IDH1-mediated epigenetic dysregulation is not easily reversed (Rohle et al., 2013). Since AGI-5198 was discovered, several other inhibitors have been identified that reduce D-2HG production in both in vitro and in vivo models (Popovici-Muller et al., 2012; Zheng et al., 2013). Shortly after the discovery of AGI-5198, medicinal chemistry optimization yielded the first inhibitor of IDH2-R140Q (AGI-6780) that reversed the hematopoietic differentiation induced by IDH2-R140Q in TF-1 erythroleukemia cells (Wang et al., 2013). Several additional inhibitors of the IDH2-R172K and IDH2-R140Q, the two highest frequency IDH2 mutations, have recently been discovered, including IDH2-C100 and AG-221, a derivative of AGI-6780, which both exhibit efficacy in cell and in vivo models (Patent WO 2013102431) (Yen et al., 2013). In aggressive IDH2 mutant primary AML xenografts models, AG-221 treatment reduced 2HG levels >90%, reversed histone and DNA hypermethylation, and conferred significant survival benefits to mice (Yen et al., 2013).

Clinical data from Phase I/II trials are emerging at a rapid rate, providing encouraging results for AML. As we continue to gain a better appreciation of the response of solid and blood tumors to these inhibitors, alternative approaches worth investigating are combinatorial treatments that target the metabolic deficiencies in IDH mutant tumors. While resistance to changes the epigenetic state of IDH mutant cells may emerge, these tumors are unlikely to regain the wild-type IDH1 or IDH2 activity that was originally lost to mutation. Therefore, pharmacological inhibition of the specific metabolic pathways on which IDH1 or IDH2 mutant cells are critically dependent may prove efficacious.

9. Conclusion

The discovery, functional characterization, and clinical development of therapies surrounding oncogenic IDH mutations highlight the great potential impact of advanced scientific technologies on medicine. In order to fully exploit the metabolic and physiological defects of IDH mutant tumors additional studies are required to identify and target such biochemical pathways in cellular and preclinical models. Improved biological models are still required, since patient-derived IDH mutant tumor cells grow slowly, ectopic expression of mutant enzymes is unstable and ineffective at producing high D-2HG levels, and isogenic, engineered cell lines lack appropriate biological context. Ultimately, molecular level analyses of how IDH mutations impact the metabolism, epigenetics, and oncogenic development of tumors will lead to additional insights into the pathogenesis of other transforming events (e.g. SDH and FH-deficient tumors) and inborn errors of metabolism (L-2HG and D-2HG aciduria).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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