Cancer-associated Isocitrate Dehydrogenase 1 (IDH1) R132H Mutation and d-2-Hydroxyglutarate Stimulate Glutamine Metabolism under Hypoxia*

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Results: IDH1 mutation increases the proportion of palmitate derived from [13C]glutamine under hypoxic conditions. Conclusion: IDH1 mutation can stimulate tumor cell reductive glutamine metabolism. Significance: IDH1 mutation may cause the unique glutamine-dependent metabolic phenotype observed in tumors.

Mutations in the cytosolic NADP⁺-dependent isocitrate dehydrogenase (IDH1) occur in several types of cancer, and altered cellular metabolism associated with IDH1 mutations presents unique therapeutic opportunities. By altering IDH1, these mutations target a critical step in reductive glutamine metabolism, the metabolic pathway that converts glutamine ultimately to acetyl-CoA for biosynthetic processes. While IDH1-mutated cells are sensitive to therapies that target glutamine metabolism, the effect of IDH1 mutations on reductive glutamine metabolism remains poorly understood. To explore this issue, we investigated the effect of a knock-in, single-codon IDH1-R132H mutation on the metabolism of the HCT116 colorectal adenocarcinoma cell line. Here we report the R132H-isosbolute by using targeted 13C isotopomer tracer fate analysis to trace the metabolic fate of glucose and glutamine in this system. We show that introduction of the R132H mutation into IDH1 up-regulates the contribution of glutamine to lipogenesis in hypoxia, but not in normoxia. Treatment of cells with a d-2-hydroxyglutarate (d-2HG) ester recapitulated these changes, indicating that the alterations observed in the knocked-in cells were mediated by d-2HG produced by the IDH1 mutant. These studies provide a dynamic mechanistic basis for metabolic alterations observed in IDH1-mutated tumors and uncover potential therapeutic targets in IDH1-mutated cancers.

Mutations in the cytosolic NADP⁺-dependent isocitrate dehydrogenase (IDH1) are frequent somatic alterations in gliomas, acute myeloid leukemias, and chondrosarcomas (1–3). IDH1 mutations have also been found at a lower frequency in colorectal adenocarcinoma and other types of cancer (4–7). IDH1 mutations, the most frequent of which is IDH1-R132H, disrupt the normal function of IDH1 to interconvert isocitrate and α-ketoglutarate (1, 8). Instead, the mutations confer a gain-of-function for IDH1 to convert α-ketoglutarate to the D isomer of 2-hydroxyglutarate (D-2HG, also known as (R)-2-hydroxyglutarate) (9). IDH1 mutations have been associated with several effects on cellular metabolism. IDH1 mutation is associated with lower levels of glutamine and other metabolites in primary glioma tissues (10, 11). Cancer cells expressing mutant IDH1 are dependent on glutamine and are sensitive to inhibition of glutaminase, presumably because D-2HG production drains glutamine to sensitize cells to deficiencies in the production of these metabolites (11–13). Also, in gliomas, IDH1 mutation is associated with silencing of the lactate dehydrogenase A (LDHA) gene and decreased glycolysis (14). The finding that IDH1 mutation drives metabolic and other alterations in cancer cell biology may have significant implications for developing therapeutic strategies to target IDH1 mutations.

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* The abbreviations used are: IDH1, cytosolic NADP⁺-dependent isocitrate dehydrogenase; IDH2, mitochondrial NADP⁺-dependent isocitrate dehydrogenase; TCA, tricarboxylic acid cycle; 2HG, d-2-hydroxyglutarate; WT, wild type; LDHA, lactate dehydrogenase A; HOG, human oligodendroglioma cell line; JC-1, 5,5′,6′,6′-tetrachloro-1,1′,3′,3′-tetratetethylbenzimidazolylcarbocyanine iodide; OCR, oxygen consumption rate.
cells has generated enthusiasm that altered metabolism in IDH-mutated tumors can be therapeutically exploited.

Reductive glutamine metabolism has recently become appreciated as a critical metabolic pathway in cancer cells. Hypoxic conditions can shift the source of cytosolic acetyl-CoA for anabolic processes away from glucose and toward glutamine in proliferating cells (15–17). Even in normoxic conditions, defects in hypoxia regulatory networks and defects in oxidative mitochondrial metabolism can shift the source for acetyl-CoA for anabolic processes toward glutamine (15–17). In these scenarios, glutamine contributes to anabolic processes via the reductive glutamine pathway, in which glutamine is converted to acetyl-CoA via glutamate, α-ketoglutarate, isocitrate, and then citrate (see Fig. 1B). Reductive carboxylation of α-ketoglutarate to form isocitrate is a critical step in reductive glutamine metabolism that is mediated by wild type IDH1 in cancer cells cultured under hypoxia, and in cancer cells with defects in hypoxia regulatory networks or with alterations in mitochondrial function (16–18). However, the effect of the cancer-associated IDH1 mutations on reductive glutamine metabolism has not been fully explored. Alterations in the steady-state levels of metabolites derived from acetyl-CoA, and of tricarboxylic acid (TCA) cycle intermediates, have been observed in cell lines ectopically expressing mutant IDH1 (10, 13). Nevertheless, whether the dynamic reductive glutamine pathway to produce acetyl-CoA or other biomolecules such as lipids is altered by IDH1 mutation under normoxia or hypoxia is unknown.

We sought to comprehensively explore the dynamic metabolic changes enforced by IDH1 mutation in cancer cells. We focused on isogenic HCT116 colorectal adenocarcinoma cell lines, which contained either a normal, wild-type IDH1 locus (IDH1WT/WT), or had a heterozygous single-codon IDH1-R132H mutation introduced by recombineering (IDH1R132H/WT) (19). We chose the HCT116 line because it provides a well-studied metabolic system to interrogate for metabolic changes introduced by knock-in IDH1-R132H mutation (20). We used multiple 13C-labeled tracers to interrogate dynamic metabolic processes in the setting of IDH1 mutation, mapping the “R132H-isobolome.” To examine the contribution of D-2HG to the metabolic phenotypes observed in IDH1-mutated cells, cells were treated with a cell-permeable ester of D-2HG. Also, we compared these systems under normoxic and hypoxic conditions, and characterized oxidative mitochondrial processes in these cell lines. This analysis revealed that the IDH1 mutation causes cancer cells to switch toward reductive glutamine metabolism under hypoxia.

FIGURE 1. Alterations in the carbon source for lipogenesis in IDH1-mutated cells under normoxia (21% O2). A, HCT116 cells with wild type IDH1 (WT/WT), with IDH1-R132H knock-in (R132H/WT), or treated for 7 days with 0.1 mM octyl-D-2HG (WT/WT + D-2HG) were incubated for 24 h in normoxia (21% O2) with [1,2-13C2]glucose. Isotopomer analysis of palmitate is shown. B, HCT116 sublines were incubated for 24 h with [1,2-13C2]glucose. Isotopomer analysis of palmitate is shown. Data are representative of two independent experiments, shown as mean ± S.D.; n = 4; *, p < 0.05; **, p < 0.001 for comparison with the paired WT/WT data.

IDH1 Mutation, D-2-Hydroxyglutarate, and Glutamine
the catalogue (0.4%). All five IDH1-mutated colorectal cancers from the COSMIC database were originally identified in either of two genome-wide sequencing analyses (4, 5). These samples include three samples with R132C mutations, one sample with an R132H mutation, and one sample with an R132G mutation. All of these IDH1 mutations confer the gain-of-function for IDH1 to produce D-2-HG (9). The fact that colorectal cancers harbor recurrent somatic mutations in IDH1 that can lead to D-2HG production, even though at a much lower frequency than other cancer types, suggests that IDH1 mutations can exert similar oncogenic and metabolic effects in colorectal cancer as in other cancer types. Therefore, we reasoned that the HCT116 colorectal cancer cell line provides a relevant context to investigate the effects of IDH1 mutation on cancer cell metabolism.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—[1,2-13C2]glucose was D-glucose-1,2-13C2 99 atom % 13C from Isotec (Miamisburg, OH, Cat No. 453188). [U-13C]Glutamine was L-glutamine-13C5 98 atom % 13C (Isotec, Cat No. 605166). [U-13C]Palmitate was sodium palmitate-13C16 98 atom % 13C (Isotec, Cat No. 605166). Octyl-D-2HG was synthesized as described previously (22) and confirmed by LC-MS.

**Cell Culture**—HCT116 sublines described previously (23), were cultured in McCoy’s 5A media (Invitrogen, Cat. No. 16600) with 10% fetal bovine serum and no antibiotics. HOG sublines described previously (10) were cultured in IMDM (Invitrogen, Cat. No. 12440) with 10% fetal bovine serum and 1% antibiotic/antimycotic (Invitrogen, Cat. No. 15240). Both lines were tested and authenticated by Sanger sequencing of IDH1 exon 4 as recently as July, 2013.

**Isotopomer Analysis**—3 × 106 cells were seeded in 10 ml of media in a 10-cm plate (n = 4). The next day, media was exchanged for 10 ml of basal DMEM without glucose, L-glutamine, and sodium pyruvate (Mediatech, Manassas, VA, Cat. No. 12-207CV) supplemented with 25 mM glucose, 4 mM glutamine, 16 μM palmitate, and 20 mM HEPES. Half of the total molar concentration of either glucose, glutamine, or palmitate was supplied as [1,2-13C2]glucose, [U-13C]glutamine, or [U-13C]palmitate, respectively. Cells were pulsed with the isotope for 24 h, either in 21% oxygen (normoxia) or in 1% oxygen (hypoxia) in a hypoxia C-Chamber (BioSpherix, Lacona, NY, Cat No. C-374). For D-2HG treatment, cells were incubated in 0.1 mM octyl-D-2HG for 7 days before treatment, and during incubation with isotope tracers.

After 24 h, media was snap-frozen on dry ice and stored at −80 °C. Cells were washed twice with PBS, scraped into 5 ml of PBS, pellets were resuspended in 5 ml of PBS, pelleted at 300 × g for 5 min at 4 °C, supernatant was removed, and pellets were snap-frozen on dry ice and stored at −80 °C. Derivatization of samples and GC-MS isotopomer analysis was performed as described (24–26). Glucose, palmitate, CO2, and lactate were quantified in the spent extracellular media. Palmitate was also quantified in the cell pellet. Data are presented after correction for natural enrichment.

**Bioenergetics Analysis**—Oxygen consumption assays were performed using the XF BioAnalyzer (Seahorse Bioscience) in 24-well plates. Oligomycin, FCCP, and rotenone from the XF Cell Mito Stress Test Kit (Seahorse Bioscience, Cat. No. 101706-100) were added as specified by the manufacturer as indicated in the figures. 5,5′,6,6′-tetrachloro-1,1′,3′,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) was applied using the MitoProbe™ JC-1 Assay Kit kit (Invitrogen, Cat. No. M34152) according to the manufacturer’s instructions with a Beckman Coulter FC500 Flow Cytometer.

**Database Reporting**—COSMIC v69 was interrogated for the frequency of IDH1 mutations in colorectal adenocarcinomas by searching the CosmicMutantExport_v69_310514.tsv table for all IDH1 R132 missense mutations in samples with both the primary site of “large intestine” and histology subtype of “adenocarcinoma.” All mutations found had unique patient age, sample ID, and tumor ID, indicating that they were all independent mutations rather than non-unique mutations reported in duplicate. The frequency of mutations was determined by dividing the number of samples with IDH1 R132 mutations by the total number of large intestine adenocarcinoma samples.
tested for mutations in IDH1 as reported in the Cancer Browser.

Statistical Analysis—For isotopomer analysis, Welch’s 2-tailed t test was used to test whether a significant difference existed between two groups. Results are expressed as means ± S.D., except where noted.

RESULTS

The R132H-Isobolome: IDH1 Mutation Increases the Contribution of Glucose to Palmitate Synthesis—We used targeted $^{13}$C isotopomer tracer fate analysis to determine the effect of IDH1 mutation on dynamic metabolic processes that generate acetyl-CoA. To investigate the carbon source of cytosolic acetyl-CoA in IDH1-mutated cells, we examined the contributions of glucose and of glutamine to new synthesis of a representative metabolite that is synthesized from acetyl-CoA: the lipid palmitate. To do so, cells were pulsed with $[1,2-^{13}$C$_2]$glucose and the relative accumulation of $^{13}$C in palmitate and other targeted metabolites was quantified. When supplied with $[1,2-$

![octyl-D-2-hydroxyglutarate](image)

FIGURE 3. Levels of $\alpha$-2HG in cells treated with octyl-$\alpha$-2-HG. $\alpha$-2HG was quantified in whole cell lysates from HCT116 cells with knock-in IDH1 mutation or with the indicated concentration of octyl-$\alpha$-2-HG.

![FIGURE 4. Targeted tracer fate associations based on $[1,2-^{13}$C$_2]$glucose tracer. $^{13}$C isotopologue data were normalized to the matched WT/WT samples (either normoxia- or hypoxia-treated). Samples included HCT116 cells with wild type IDH1 (WT/WT), with IDH1-R132H knockin (R132H/WT), or octyl-$\alpha$-2HG-treated cells (WT/WT + $\alpha$-2HG), incubated under either normoxia or hypoxia. The Pearson product moment correlation coefficient and R squared values were determined for comparisons to $^{13}$C$_2$ production (total oxidation) data.](image)
tate species as shown in Fig. 1A. IDH1R132H/WT cells contained a higher fraction of palmitate with labeled 2-carbon units (Fig. 1A). For example, the fraction of m/10012 palmitate was increased 90% in IDH1R132H/WT cells (p/10012). This difference demonstrates that IDH1-mutated cells incorporated glucose into new palmitate to a greater extent than wild type controls.

To determine the effect of IDH1 mutation on reductive glutamine metabolism, we traced the fate of13C nuclei from uniformly 13C-labeled glutamine ([U-13C]glutamine) in IDH1R132H/WT cells. Through reductive carboxylation, glutamine can be converted to acetyl-CoA and used to donate 2-carbon units for new palmitate synthesis. Because [1,2-13C2]glucose and [U-13C]glutamine each yield each one labeled acetyl-CoA that can contribute to new palmitate synthesis, comparing palmitate labeling between cells pulsed with [1,2-13C2]glucose and [U-13C]glutamine can provide information on the carbon source for palmitate. No major difference was observed in palmitate labeling by glutamine in IDH1R132H/WT cells (Fig. 1B). Together, these data indicate that, in the setting of hypoxia in IDH1-mutated cells, the source of acetyl-CoA for lipogenesis shifts from glucose to glutamine.

The 2HG-isobolome: D-2HG Phenocopies Knock-in IDH1 Mutation—We next sought to determine whether D-2HG is sufficient to cause any of the metabolic alterations elicited by IDH1 mutation. To simulate intracellular levels of D-2HG comparable to those produced by IDH1 mutations, we used the cell-permeable octyl ester of D-2HG (22). Treatment with 0.1 mM octyl-D-2HG achieved the same level of intracellular D-2HG as produced in knock-in IDH1 mutant cells (Fig. 3). Along with IDH1WT/WT and IDH1R132H/WT cells, Figs. 1, 2, and 4 display data from IDH1WT/WT cells treated with 0.1 mM octyl-D-2HG (the 2HG-isobolome). To compare the metabolic alterations enforced by R132H mutation and by D-2HG treatment, we examined the Pearson correlations between targeted tracer rates for both glucose and glutamine tracers (Figs. 4 and 5).
these experiments, D-2HG phenocopied the effects of IDH1 mutation. As was the case for the knock-in IDH1 mutation, octyl-D-2HG elicited increases in lipid labeling from [1,2-\(^{13}\)C\(_2\)]glucose (Fig. 1A) that were attenuated under hypoxia (Fig. 2A). Also, under hypoxic conditions, D-2HG treatment resulted in increases in the fraction of m+2, m+4, m+6 palmitate derived from [U-\(^{13}\)C]glutamine (Fig. 2B). These data suggest that D-2HG mediates the switch toward glutamine as a carbon source for lipogenesis in IDH1-mutated cells under hypoxia.

Our results indicated that, in the setting of IDH1 mutation or treatment with exogenous D-2HG, the contribution of glucose to lipogenesis under normoxia was increased. The increase results from entry of glucose-derived pyruvate into the mitochondria to ultimately support lipid synthesis via the citrate shuttle. Under hypoxia, the source of carbon for new lipid synthesis in IDH1-mutated and D-2HG-treated cells shifted from glucose toward glutamine, reminiscent of cells with defective mitochondria (15–18). Together, these results led us to investigate mitochondrial function in HCT116 cells with knock-in IDH1 mutation.

**IDH1 Mutation and Total Oxidation of Metabolites—** We next investigated the total oxidation of nutrients to CO\(_2\), a process that primarily occurs in the mitochondrial oxidative TCA (Fig. 6A). The fraction of CO\(_2\) that was released into the media that was 13C-labeled (\(^{13}\)CO\(_2\)) when cells were pulsed with [1,2-\(^{13}\)C\(_2\)]glucose was 3.7-fold increased by knock-in IDH1 mutation, indicating increased entry and cycling of glucose-derived carbon into the TCA (\(p < 10^{-5}\), Fig. 6B). D-2HG also elicited a 2.1-fold increase in the proportion of [1,2-\(^{13}\)C\(_2\)]glucose-derived \(^{13}\)CO\(_2\) (\(p < 10^{-5}\)). Similar increases in this oxidative fate for glucose were observed in hypoxic conditions (Fig. 6C).

As was the case for glucose, IDH1 mutation or D-2HG treatment resulted in a \(>2\)-fold increase in the proportion of CO\(_2\) labeled by [U-\(^{13}\)C]glutamine under normoxic conditions (\(p < 10^{-5}\) for each comparison, Fig. 6D). However, this difference was attenuated under hypoxia, to less than a 1-fold increase in labeling caused by either IDH1 mutation knock-in or D-2HG (Fig. 6E). This result is in line with the increased burden for glutamine to contribute to acetyl-CoA production for lipogenesis, reducing its contribution to the generation of TCA intermediates for total oxidation. IDH1 mutation additionally increased the contribution of exogenous [U-\(^{13}\)C]palmitate to CO\(_2\), indicating lipids also undergo increased total oxidation in the TCA (Fig. 6F).

**Glycolysis in IDH1 Knock-in Cells—** Our results suggested that more glucose-derived pyruvate was entering the mitochondria in IDH1-mutated cells, leading to an increased contribution of glucose to CO\(_2\) via total oxidation in the TCA and increased contribution of glucose to lipid synthesis via the cit-
rate shuttle. This increase of glucose entry to the mitochondria could result from increased glucose uptake and glycolysis to provide substrates to the TCA, or it could reflect that the ratio of pyruvate entering the TCA compared with fermentation to lactate was altered. Glucose uptake was increased slightly in IDH1R132H/WT cells (Fig. 7A).

Next, we assayed dihydrolipoamide dehydrogenase activity, a marker for entry of glycolysis-derived pyruvate into the mitochondria via pyruvate dehydrogenase. Dihydrolipoamide dehydrogenase activity was up-regulated in two IDH1R132H/WT sublines (Fig. 7B). The amount of lactate m+2 labeling reflects the extent to which [1,2-13C2]glucose underwent glycolysis to generate lactate. The m+2 lactate fraction was only slightly increased in IDH1R132H/WT cells (Fig. 7, C and D). The large increase in dihydrolipoamide activity, together with the minimal change in lactate labeling, indicate that the increased glucose-derived 13CO2 fraction observed in mutant IDH1 knock-in cells (see Fig. 2A) was due to oxidation of TCA intermediates that were derived from entry of glycolytic pyruvate into the TCA.

Mitochondrial Function in IDH1-mutated Cells—The metabolic alterations reflected by our isotope labeling experiments are summarized in Fig. 8A. Taken together, these tracer experiments indicate that total oxidation of metabolites in the TCA is increased in IDH1-mutated cells. These alterations were likely caused by D-2HG, since exogenous D-2HG enforced similar changes. Glucose uptake and glycolysis were increased in IDH1-mutated cells to support these alterations. The increases in labeling of CO2 in IDH1-mutated cells were maintained even under hypoxia. This is surprising because under hypoxia, total oxidation of TCA intermediates to provide substrates for the electron transport chain, and oxidative phosphorylation would be expected to be lowered because limited O2 is available to act as a final electron acceptor for these processes.

To explore this issue, we assessed oxygen consumption at baseline, in the condition of no oxidative phosphorylation as monitored in the presence of oligomycin, under the condition of maximal electron transport by treating cells with the uncoupling agent FCCP, and in the setting of electron transport inhibition by treating cells with rotenone. Despite increased TCA cycling observed in our tracer experiments, no significant difference was observed between the oxygen consumption rate of mutant and wild type HCT116 cells (Fig. 8B). In line with this observation, no significant differences were noticed in ATP levels in these HCT116 cell lines (Fig. 8C). The increase in total oxidation of TCA intermediates but absence of a corresponding oxygen consumption increase suggested a defect in the electron transport chain (ETC) to shuttle electrons to oxygen. An intact ETC is needed regulate the negative charge potential at the inner mitochondrial membrane. To assess this potential, we assessed the ratio of red/aggregated and green/monomeric forms of JC-1 dye in cells. This analysis revealed an increased JC-1 red:green ratio in IDH1-mutated HCT116 cells, reflecting an increase in the absolute inner mitochondrial membrane potential (Fig. 9A). Similarly, an increased JC-1 red:green ratio was observed in a human oligodendroglioma cell line (HOG) subline stably overexpressing mutant IDH1 (10).
compared with an HOG subline treated with vector alone (Fig. 9B).

**DISCUSSION**

This study is the first metabolomics analysis of a single-codon knock-in IDH1 mutation model. This model more faithfully recapitulates the genetic situation in human IDH1-mutated cancer cells than systems that overexpress mutant IDH1, or systems that do not compare IDH1-mutated cells to an isogenic control. The current study is also the first comprehensive analysis of targeted isotope tracer fates (the R132H-isobolome) and bioenergetics profiles for IDH1-mutated cancer cells. These analyses revealed that IDH1 mutation increases entry of carbon from glucose and glutamine nutrients for TCA cycling and for biosynthetic processes such as lipogenesis, as reflected by new palmitate synthesis. Under hypoxia, a shift from glucose to glutamine as a lipogenic carbon source was only seen in cells with a knocked-in IDH1 mutation and not in cells without IDH1 mutation.

IDH1 mutations likely caused the above metabolic effects through d-2HG, since addition of a d-2HG ester phenocopied almost all of the metabolic changes associated with IDH1 mutation. We cannot rule out the possibility that processing of cells incubated with octyl-d-2HG for quantification of free d-2HG could lead to hydrolysis of the octyl ester, thus overestimating the intracellular concentration of free d-2HG (Fig. 3). However, even if the amount of free d-2HG added to cells was overestimated, our experiments demonstrate that d-2HG elicits the same directionality of changes as a knocked-in IDH1 mutation.

The shift toward glutamine-supported lipogenesis in IDH1-mutated and d-2HG-treated cells is reminiscent of the phenomenon observed in proliferating cells with mitochondrial dysfunction. This effect has been reported in cells with cytochrome C reductase dysfunction, in cells with disrupted hypoxia signaling pathways, and in cells cultured under hypoxia, all of which have been identified as etiologies for altered mitochondrial function (15–18). A plausible explanation for the similarity between metabolic alterations caused by IDH1 mutation knock-in and by mitochondrial dysfunction is that the d-2HG produced by mutant IDH1 can cause mitochondrial dysfunction by inhibiting complexes I, IV, and V of the electron transport chain (27, 28). Indeed, our JC-1 dye data indicate that electron transport chain function is altered in IDH1-mutated cells. Another possible contributor to this metabolic shift is the ability for d-2HG to activate EGLN prolyl hydroxylases to degrade hypoxia inducible factors, which regulate metabolic processes in normal and hypoxic conditions (23).
We have attributed increases in the fraction of palmitate labeled by glutamine to increased net reductive glutamine metabolism. It is important to note that increases in the fraction of palmitate labeled by glutamine could also be explained by isotope exchange. This caveat to the interpretation of glutamine isotope tracer data has recently been explored with a quantitative flux model (29). Since all exchanges in the pathway between glutamine and citrate are reversible, the citrate pool could accumulate label from glutamine even if the pathway has an even higher flux in the oxidative direction. Thus, citrate and palmitate could still be labeled by glutamine even if net flux along the pathway between glutamine and citrate were in the overall oxidative direction. In this scenario, glutamine-labeled citrate would dilute the pool of citrate derived from glucose to ultimately yield increased labeled palmitate, even though net reductive glutamine flux is not increased per se.

FIGURE 9. Mitochondrial membrane potential in cell lines with knocked-in IDH1 mutation or stable expression of IDH1 mutation. A, JC-1 staining of HCT116 cells. Flow cytometry plots for JC-1 red and JC-1 green staining in IDH1 mutation knock-in sublines of HCT116 (R132H/WT #1 and R132H/WT #2) compared with IDH1 wild type subline. Relative mean JC-1 intensity is quantified in plot below. B, JC-1 staining of human oligodendroglioma cell line (HOG). Flow cytometry plots for JC-1 red and JC-1 green staining in HOG cell subline stably expressing IDH1-R132H, or a subline treated with a vector control. Relative mean JC-1 intensity is quantified in plot below.
Thus, the present study and other recent studies that noted increased labeling of lipids by glutamine could reflect isotope exchange in this manner, increased net reductive glutamine metabolism, or both (15–17). Nevertheless, the observation of palmitate labeled by glutamine unambiguously demonstrates increased activity by higher even number. $^{13}$C positions of the glutamine pathway in the reductive anabolic direction. The observation of increases in the proportion of palmitate labeled by glutamine strongly imply that a shift toward reductive glutamine metabolism has occurred in IDH1-mutated cells under hypoxia (this study) and in cells with defective mitochondria or hypoxia regulatory networks (15–17), even if an increase in net reductive glutamine flux cannot be directly assumed.

Our study revealed a dramatic contrast between the effects of the cancer-associated IDH1-R132H point mutation and knockdown of wild type IDH1 and/or knockdown of wild type IDH2 (the mitochondrial homolog of IDH1) (15, 16). Reductive glutamine metabolism was attenuated when IDH1 or IDH2 was knocked down in cancer cells with defects in hypoxia signaling or electron transport chain function (15–17). However, we show that IDH1-R132H mutation has the opposite effect to IDH1/2 knockdown, in that the IDH1 mutation enhances reductive glutamine metabolism under hypoxia. This observation has several implications. First, the present data show that, in IDH1<sup>R132H/WT</sup> cells, the single wild type IDH1 allele is sufficient to support reductive glutamine processes. Thus, only a fraction of total wild type IDH1 reductive carboxylation turnover capacity may be needed for to support reductive glutamine metabolism. The finding that wild type IDH1 does not act as a “bottleneck” for glutamine metabolism is consistent with previous data showing that cells expressing mutant IDH1 were susceptible to glutaminase inhibition due to a “bottleneck” at the earlier glutaminase step (13). The contrasts between IDH1 mutation and knockdown of IDH1 provide a functional distinction between the R132H mutations observed frequently in cancer and loss of function mutations, which are rarely observed in IDH1 in cancer.

Introduction of a single-codon mutation to a tractable metabolic system demonstrates that IDH1 mutations have a metabolic rewiring function that helps to provide building blocks for the proliferation of tumor cells. Metabolic rewiring is also a result of cancer-associated alterations in other genes such as KRas, PIK3CA, and Myc (30–32). Thus, metabolic reprogramming is a common feature shared by IDH1 mutations and other oncogenes, although the mechanism by which metabolic alterations are enforced may be different for each alteration. Several features of the metabolic effects of IDH1 mutation, including lowered steady-state levels of the TCA metabolites malate and fumarate (10), are also reminiscent of cancer-associated loss-of-function mutations in fumarate hydratase (FH) and in succinate dehydrogenase (SDH) that result in a blockade of the oxidative TCA (33, 34).

We used the HCT116 colorectal cancer cell line as our primary model system in this study since the metabolic properties of this line have been thoroughly investigated previously (20), and since this cell line is amenable to genetic manipulation (19). Since IDH1 mutations occur rarely in colorectal tumors (4, 5), it will be critical to develop knock-in IDH1 mutation model systems derived from cancer types that more typically harbor IDH1 mutations, such as gliomas and acute myeloid leukemias. Study of such models will allow further insight into the effect of IDH1 mutation in the context of the cancer types in which such mutations are frequently selected, and provide information to guide the development of therapeutics for specific cancer types.

The current study maps the effects of IDH1 mutations and of D-2HG on cancer cell metabolism. These data reveal that IDH1 mutation up-regulates reductive glutamine metabolism to generate new lipids, and maps reductive glutamine processes that are under investigation as therapeutic interventions (13). Validation of components of these pathways as therapeutic targets will involve testing whether disrupting those components can attenuate tumor cell growth in faithful models of IDH1-mutated tumors.

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