Fatty Acid Labeling from Glutamine in Hypoxia Can Be Explained by Isotope Exchange without Net Reductive Isocitrate Dehydrogenase (IDH) Flux

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Background: Cancer cells in hypoxia were claimed to rely on reductive isocitrate dehydrogenase (IDH) for lipogenesis based on increased isotopic labeling of fatty acids from glutamine.

Results: Oxidative IDH flux persists in hypoxia, whereas acetyl-CoA demand drops.

Conclusion: Reductive IDH flux may not be a net contributor to acetyl-CoA production.

Significance: Isotopic label incorporation is indicative of flux, although not necessarily of net flux.

Acetyl-CoA is an important anabolic precursor for lipid biosynthesis. In the conventional view of mammalian metabolism, acetyl-CoA is primarily derived by the oxidation of glucose-derived pyruvate in mitochondria. Recent studies have employed isotope tracers to show that in cancer cells grown in hypoxia or with defective mitochondria, a major fraction of acetyl-CoA is produced via another route, reductive carboxylation of glutamine-derived α-ketoglutarate (catalyzed by reverse flux through isocitrate dehydrogenase, IDH). Here, we employ a quantitative flux model to show that in hypoxia and in cells with defective mitochondria, oxidative IDH flux persists and may exceed the reductive flux. Therefore, IDH flux may not be a net contributor to acetyl-CoA production, although we cannot rule out net reductive IDH flux in some compartments. Instead of producing large amounts of net acetyl-CoA reductively, the cells adapt by reducing their demand for acetyl-CoA by importing rather than synthesizing fatty acids. Thus, fatty acid labeling from glutamine in hypoxia can be explained by spreading of label without net reductive IDH flux.

Even in the presence of adequate oxygen, tumor cells manifest up-regulated glycolysis relative to oxidative phosphorylation (Warburg effect) (6). In most cases, this is due to increased glycolysis that is induced by oncogene signaling rather than impairment of mitochondrial function (7). In some tumors, however, mitochondrial function is impaired by mutated mitochondrial proteins. For example, somatic mutations in the TCA cycle genes fumarate hydratase and succinate dehydrogenase are tumorigenic (8). In both cases, the mutations lead to the activation of HIF, which causes a pseudohypoxic state, resulting in similar phenotypes to those of hypoxic cells even in the presence of oxygen.

An important intermediate of oxidative mitochondrial metabolism is acetyl-CoA. Cytosolic acetyl-CoA is the main precursor for de novo fatty acid biosynthesis. The canonical pathway for production of cytosolic acetyl-CoA begins with the oxidation of glucose-derived pyruvate in mitochondria (see Fig. 1). The resulting mitochondrial acetyl-CoA is consumed by citrate synthase to convert oxaloacetate into citrate. Citrate may then be either oxidized in TCA cycle or shuttled to cytoplasm, where its cleavage by ATP citrate lyase produces cytosolic acetyl-CoA (9).

Recent studies have employed isotope tracing to study how acetyl-CoA is produced in mammalian cell by feeding with 13C-labeled glutamine, glutamate, or succinate and measuring the resulting labeling of citrate and fatty acids (10–16). Experiments in liver and cardiac cells established that a fraction of citrate and fatty acid 2-carbon units originates from α-ketoglutarate through reductive carboxylation of isocitrate dehydrogenase (IDH) (14–16). More recently, reductive IDH was claimed to be particularly important in hypoxia and pseudohypoxia, where a major fraction of fatty acid carbon units originates from glutamine (10–13).

Although these isotope tracer experiments unambiguously demonstrate reverse IDH flux, they do not address the question of whether there is actually net flux in the reductive direction. In general, isotope labeling patterns reflect gross (i.e. total) flux in a given direction, which may be offset by yet greater flux in...
Fatty Acid Labeling from Glutamine in Hypoxia

the opposite direction and not necessarily net conversion. This key principle was elucidated more than half a century ago to refute claims that liver synthesizes glucose from fatty acids based on the experimental observation that feeding cells with labeled fatty acids results in glucose labeling (17, 18). More recently, Landau and Wahren (19) have emphasized the difference between label incorporation and net flux in the context of identification of pseudoketogenesis, which can be misinterpreted to grossly overestimate ketogenesis (20–22).

In this work, we employ a quantitative flux model to examine oxidative and reductive IDH flux in cancer cells grown in hypoxia and in cells with defective mitochondria. We analyze the lung cancer cell line A549 grown in hypoxia and the osteosarcoma cell line 143B-CYTb with a defective electron transport chain. In both cell types, reductive IDH flux was recently claimed to have a central role in lipogenesis (10, 11). We show that the observed fatty acid labeling from glutamine does not necessarily imply net reductive IDH flux. Indeed, by placing analytical bounds on the oxidative and reductive IDH fluxes based on metabolite isotope labeling, we find evidence for oxidative net flux in pseudohypoxia and for modest or no net flux in either direction in hypoxia. Thus, reductive IDH flux is not a major net contributor to acetyl-CoA production. Instead, cells cope with limited oxidative acetyl-CoA production by reducing the biosynthetic utilization of acetyl-CoA for fatty acid synthesis (23).

EXPERIMENTAL PROCEDURES

Cell lines were grown in Dulbecco’s modified Eagle’s media (DMEM) without pyruvate (Cellgro), supplemented with 10% dialyzed fetal bovine serum (HyClone). Isogenic 143B human osteosarcoma cells that contained (143B-CYTb) or lacked (143B-WT) a loss-of-function mutation in mitochondrial complex III were grown in an incubator containing 5% CO2 and ambient oxygen at 37 °C. For hypoxia experiments, A549 cells were grown inside a hypoxic chamber (Coy Laboratory Products) containing 1% oxygen and 5% CO2 at 37 °C. For labeling experiments, medium was prepared from DMEM without glutamine (Cellgro), with the desired isotopic form of glutamine added to a final concentration 0.584 g/liter. Metabolite extractions were conducted at 70–80% confluency.

For all metabolomic and isotope tracer experiments, metabolism was quenched, and metabolites were extracted by quickly aspirating media and immediately adding −80 °C 80:20 methanol:water extraction solution. Samples were analyzed for water-soluble metabolites and saponified fatty acids by a stand-alone orbitrap mass spectrometer (Exactive) operating in negative ion mode and coupled to reversed-phase ion-pairing chromatography as described previously (23, 24). In addition, confirmatory measurements of water-soluble metabolites were acquired with a TSQ Quantum Discovery triple-quadrupole mass spectrometer operating in negative ion, multiple-reaction monitoring mode coupled to reversed-phase ion-pairing chromatography as described (25). Data were analyzed using the MAVEN software suite (26). Metabolite labeling patterns were adjusted for natural 13C abundance and for enrichment impurity of labeled substrate. Absolute metabolite levels were quantified as described previously (27) and normalized by packed cell volume. Acetate secretion rate was measured using the acetate (ACS manual format) test kit (Megazyme; catalogue number K-ACET) according to manufacturer’s instructions. Oxygen consumption was measured by a Seahorse XF24 flux analyzer (Seahorse Bioscience, North Billerica, MA). To measure oxygen uptake in hypoxia, the Seahorse instrument was placed in the hypoxia chamber with 1% oxygen.

RESULTS

Analysis of Feasible IDH Fluxes Based on the Experimentally Observed Acetyl-CoA Labeling from [13C]Glutamine—Although previous studies have shown that a major fraction of cytosolic acetyl-CoA gets labeled from [U-13C]glutamine in hypoxia and pseudohypoxia (10–13), it is possible that oxidative IDH persists and exceeds the reductive flux. Here, we employ a simple isotopomer model to probe the feasible net IDH fluxes based on experimental measurements of TCA cycle fluxes and effluxes (of α-ketoglutarate from glutamine and of 2-carbon units) and based on acetyl-CoA labeling from [U-13C]glutamine.

Assuming pseudo steady-state patterns for metabolite labeling, the isotopomer balance model shown in Fig. 1 results in the following balance equation for citrate m + 5

\[ v3X_{\text{akgs}} = (v2 + v5)X_{\text{cit5}} \]

and α-ketoglutarate m + 5

\[ v2X_{\text{cit5}} + v4 = (v3 + v6)X_{\text{akgs}} = (v2 + v4)X_{\text{akgs}} \]

where \( X_{\text{akgs}} \) and \( X_{\text{cit5}} \) represent the fractional abundance of α-ketoglutarate m + 5 and citrate m + 5, respectively. The left-hand side in both equations represents biosynthetic routes, whereas the right-hand side represents consumption. Given experimental measurements of glutamine flux into TCA cycle via αKG (v4), acetyl-CoA demand for fatty acid biosynthesis (v5), labeling of citrate m + 5, and unidirectional reductive IDH flux (v3), Equations 1 and 2 enable computation of net oxidative IDH flux (v2 − v3). For simplicity, in this section, we assume zero labeling of mitochondrial acetyl-CoA from glutamine (i.e. negligible mitochondrial malic enzyme flux). This assumption tends to favor net flux in the reductive direction; hence, given

\[ \text{Glucose} \]

\[ \text{Pyruvate} \]

\[ \text{Ac-CoA[m]} \]

\[ \text{OAA} \]

\[ \text{Malate} \]

\[ \text{Succinate} \]

\[ \text{AKG} \]

\[ \text{Glutamine} \]

\[ \text{OAA} \]

\[ \text{Citrate} \]

\[ \text{Ac-CoA[c]} \]

\[ \text{OAA} \]

\[ \text{Citrate} \]

\[ \text{Ac-CoA[c]} \]

\[ \text{OAA} \]

\[ \text{Citrate} \]

\[ \text{Ac-CoA[c]} \]

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\[ \text{Citrate} \]

\[ \text{Ac-CoA[c]} \]

\[ \text{OAA} \]

\[ \text{Citrate} \]

\[ \text{Ac-CoA[c]} \]
that our conclusions nevertheless show a propensity for net oxidative flux, it only strengthens these conclusions. Under this assumption, there is no contribution of citrate synthase to making citrate $\Delta\text{citrate}^5$, and the abundance of cytosolic acetyl-CoA $\Delta\text{acetyl-CoA}^2$ equals that of citrate $\Delta\text{citrate}^5$.

Next, we applied this model to examine IDH fluxes in a lung cancer cell line A549 grown in hypoxia (10) and the osteosarcoma cell line 143B-CYTB having a defective electron transport chain and its isogenic wild-type cell line 143B (143B-WT) (11). Glutamine flux into TCA cycle ($\Delta\text{glutamine}^4$), as measured based on glutamine uptake rate from medium, minus glutamate and proline secretion, glutamine demand for protein biosynthesis, and dilution of glutamine and glutamate pools, was found to be 26–35 nmol/μL of cells/h across the three cell lines (Fig. 2a).

To quantify the rate of acetyl-CoA demand for de novo fatty acid biosynthesis, we measured the total cellular fatty acid concentration (free and lipid-incorporated), the fraction of fatty acid that is de novo synthesized (measured by monitoring fatty acid labeling in cells fed with both $[\text{U-}{}^{13}\text{C}]\text{glucose}$ and $[\text{U-}{}^{13}\text{C}]\text{glutamine}$), and cellular growth rate (23) (Fig. 2b). Overall, the acetyl-CoA demand for fatty acid biosynthesis is $0.55–2.24$ nmol/μL of cells/h across the three studied cell lines (Fig. 2c).

To derive an upper bound on the additional acetyl-CoA demand for protein acetylation, we computed the acetyl-CoA requirement for acetylating all proteomic lysine residues (assuming that protein amounts to 50% of cellular weight and that the frequency of lysine is 5%), which is $0.5–0.9$ nmol/μL of cells/h (Fig. 2c). To examine whether an additional demand for acetyl-CoA exists due to protein acetylation and deacetylation cycling, we measured acetate secretion to the media (28). We find that in all three cell lines, acetate secretion is negligible (smaller than 0.02 nmol/μL of cells/h). Thus, to the extent that protein acetylation cycling occurs, it is not a major net consumer of 2-carbon units.

Given these measurements of glutamine intake to TCA cycle ($\Delta\text{glutamine}^4$) and acetyl-CoA demand ($\Delta\text{acetyl-CoA}^5$) in 143B-CYTB, we applied Equations 1 and 2 to compute the IDH net flux for various combinations of hypothetical cytosolic acetyl-CoA $\Delta\text{acetyl-CoA}^2$ labeling and unidirectional reductive IDH flux ($\Delta\text{v3}^\text{+}$) (Fig. 3a). We find that when feeding $[\text{U-}{}^{13}\text{C}]\text{glutamine}$, for a given IDH net flux ($\Delta\text{v2}^\text{+}$), the fraction of acetyl-CoA $\Delta\text{acetyl-CoA}^2$ increases as the reductive IDH flux ($\Delta\text{v3}^\text{+}$) increases, asymptotically reaching $\Delta\text{v4}^\text{+}/(\Delta\text{v4}^\text{+}+\Delta\text{v1}^\text{+})$ when the citrate and αKG pools are completely mixed. When IDH net flux is oxidative ($\Delta\text{v2}^\text{+}$ > $\Delta\text{v3}^\text{+}$), mass-balance considerations entail that $\Delta\text{v1}^\text{+} > \Delta\text{v5}^\text{+}$, and hence the maximal fraction of acetyl-CoA $\Delta\text{acetyl-CoA}^2$ is bounded by $\Delta\text{v4}^\text{+}/(\Delta\text{v4}^\text{+}+\Delta\text{v5}^\text{+})$, which is between 0.85 and 0.97 in the cell lines studied here (Fig. 3b). To measure the actual cytosolic...
Fatty Acid Labeling from Glutamine in Hypoxia

FIGURE 3. Extensive labeling of acetyl-CoA from glutamine can occur without net reductive IDH flux. a, fraction of cytosolic acetyl-CoA m+2 labeling from [U-13C]glutamine (y axis) for various combinations of net IDH flux (represented by color) and unidirectional reductive IDH flux (x axis) (fluxes shown in nmol/μl of cells/h). Oxidative IDH net flux is shown in blue, whereas reductive IDH net flux is shown in green. The solid red line represents an upper bound on acetyl-CoA m+2 labeling when the net IDH flux is oxidative. The analysis was done based on measurements in 143B-CYTB. b, measured acetyl-CoA m+2 labeling (blue) versus the feasible upper bound (red) assuming oxidative IDH net flux. Error bars designate mean ± S.D.

acetate-CoA m+2 in these cell lines, we quantified fatty acid labeling from [U-13C]glutamine by LC-MS (23) and used isotopomer spectral analysis (29). We find that in all cases, the fraction of cytosolic acetyl-CoA m+2 is substantially lower than the feasible upper bound consistent with net oxidative IDH flux. Thus, net IDH flux in the oxidative direction is possible in all three cell lines (Fig. 3b).

Quantifying IDH Oxidative and Reductive Flux via Isotopic Labeling of Intracellular Metabolites—To determine the actual direction of net IDH flux, we derive analytical bounds on oxidative and reductive IDH flux based on experimentally observed metabolite steady-state labeling patterns. For this analysis, we no longer rely on the simplifying assumption of negligible labeling of mitochondrial acetyl-CoA from glutamine. Thus, we must account for citrate synthase potentially making citrate m+5. The resulting balance equation for citrate m+5 is

\[ v_3 X_{\text{akg5}} + v_1 X_{\text{mal3}} X_{\text{sc2}} = (v_2 + v_5) X_{\text{cit5}} \]  
(Eq. 3)

where \( X_{\text{mal3}} \) and \( X_{\text{sc2}} \) represent the fractional abundance of malate m+3 and mitochondrial acetyl-CoA m+2, respectively. Here, we assume rapid exchange between oxaloacetate and malate (which is easier to detect via LC-MS analysis) and hence a similar labeling pattern of both. Citrate m+5, whose only carbon 6 is unlabeled (denoted \( X_{\text{cit5,RC}} \)), is made through reductive carboxylation of \( \alpha \)-ketoglutarate m+5 and potentially through citrate synthase, depending on the positional labeling of malate m+3. Writing the balance equation for \( X_{\text{cit5,RC}} \) while omitting the undetermined contribution of citrate synthase, gives

\[ v_3 X_{\text{akg5}} = X_{\text{cit5,RC}} (v_2 + v_5) \]  
(Eq. 4)

which combined with Equation 3 (and assuming \( v_1 = v_5 = v_2 = v_3 \) due to mass-balance considerations) gives the following lower bound on \( X_{\text{cit5,RC}} \):

\[ X_{\text{cit5}} - X_{\text{mal3}} \times X_{\text{sc2}} \leq X_{\text{cit5,RC}} \]  
(Eq. 5)

The balance equation for \( \alpha \)-ketoglutarate m+5 is

\[ v_2 (X_{\text{cit5,RC}} + X_{\text{cit6}}) + v_4 = (v_3 + v_6) X_{\text{akg5}} = (v_2 + v_4) X_{\text{akg5}} \]  
(Eq. 6)

which gives

\[ v_2 = \frac{v_4}{X_{\text{akg5}} - X_{\text{cit5,RC}} - X_{\text{cit6}}} \]  
(Eq. 7)

Taken together, Equations 5 and 7 give a lower bound on oxidative IDH flux (v2).

\[ v_2 \geq v_4 (\frac{1 - X_{\text{akg5}}}{X_{\text{akg5}} - X_{\text{cit5}} - X_{\text{cit6}}}) \]  
(Eq. 8)

This derivation relies on the fact that the fraction of cytosolic acetyl-CoA that is m+2 (\( X_{\text{sc2}}^c \)) exceeds that in the mitochondrion because cytosolic citrate (the source of cytosolic acetyl-CoA, assuming negligible acetyl-CoA synthetase activity because there is no acetate in the medium) has only two sources: mitochondrial citrate (which will give equivalent labeling to the mitochondrion) or cytosolic \( \alpha \)-ketoglutarate (which will give more labeling than the mitochondrion).

Because cit5_RC is a specific isotopomer of citrate m+5, its abundance, \( X_{\text{cit5,RC}} \), is no larger than \( X_{\text{cit5}} \). Hence, based on Equation 7, we get the following upper bound on \( v_2 \)

\[ v_2 \leq v_4 (\frac{1 - X_{\text{akg5}}}{X_{\text{akg5}} - X_{\text{cit5}} - X_{\text{cit6}}}) \]  
(Eq. 9)

which combined with Equation 3 gives an upper bound on reductive IDH flux (v3).

\[ v_3 \leq v_4 (\frac{1 - X_{\text{akg5}}}{X_{\text{akg5}} - X_{\text{cit5}} - X_{\text{cit6}}} + v_5) \]  
(Eq. 10)

To employ Equations 8 and 10 to derive bounds on the oxidative versus reductive IDH fluxes, we measured by LC-MS the labeling pattern of citrate, \( \alpha \)-ketoglutarate, and malate (Fig. 4a). In both the wild-type and the mitochondrial defective 143B cell
lines, the oxidative IDH flux is significantly higher than the reductive flux, being at least 30-fold higher in 143B-WT and 2-fold higher in 143B-CYTB (Fig. 4b). In A549 grown in hypoxia, the lower bound on oxidative IDH flux is roughly the same as the upper bound on the reductive flux, implying that there is at most modest net flux in the reductive direction (Fig. 4b). Notably, considering the potential existence of other glutamine-consuming pathways not quantified here, glutamine flux into the TCA cycle may be overestimated in this study. However, as shown in Fig. 4c, our results regarding no major IDH net flux still hold for substantially lower glutamine flux into TCA cycle via α-ketoglutarate.

Mechanisms of Redox and 2-Carbon Unit Balancing in Hypoxia and Pseudohypoxia—To assess how redox balancing is achieved in hypoxia without reductive IDH net flux, we measured the oxygen consumption in A549 and its fraction used by oxidative phosphorylation (using respiratory chain inhibition). We find that the oxygen consumption rate is ~60 nmol/μl of cells/h, which suffices to account for the observed oxidative TCA flux, given the that glutamine flux to α-ketoglutarate is ~35 nmol/μl of cells/h and that glutamine is only partially oxidized in TCA cycle.

In 143B-CYTB, we find that redox balance is maintained by a branched TCA cycle activity, in which succinate is made both through the oxidation of acetyl-CoA and through the reduction of oxaloacetate. Specifically, the succinate secretion rate increases from an undetected level in 143B to ~11 nmol/μl of cells/h in 143B-CYTB. Consistent with reductive production of succinate from oxaloacetate, we observed higher pyruvate carboxylase activity in 143B-CYTB, with unlabeled malate (in cells fed [U-13C]glutamine and unlabeled glucose), increasing from 35% in 143B to 75% in 143B-CYTB.

These observations left open the question of how cells compensate for reduced acetyl-CoA production by pyruvate dehydrogenase in hypoxia. Based on recent evidence for increased fatty acid scavenging in hypoxia (23), we considered the possibility that this might serve to reduce 2-carbon unit demand. Indeed, quantitative analysis reveals that A549 cells in hypoxia decrease total 2-carbon unit consumption for fatty acid biosynthesis by 73%. Moreover, reexamination of the fatty acid labeling patterns in Fig. 2b revealed a large increase in the unlabeled fatty acid peak in cells fed with both [U-13C]glucose and [U-13C]glutamine in 143B-CYTB versus 143B-WT cells (i.e. in pseudohypoxia). Given that absolute total fatty acid concentra-
Fatty Acid Labeling from Glutamine in Hypoxia

integrations are roughly the same in both cell lines (e.g. palmitate is 18.7 ± 1.0 and 15.1 ± 2.6 mmol/µL of cells in 143B-WT and 143B-CYTb, respectively), the higher fraction of palmitate m+0 in 143B-CYTb implies enhanced fatty acid scavenging. Integrating across all experimentally measured fatty acids and accounting for cellular doubling time, we obtain a decrease in the 2-carbon units requirement of 75%.

DISCUSSION

Several recent studies have employed isotopic tracers to investigate how acetyl-CoA is produced in cancer cell lines in hypoxia and with defective mitochondria (10–13). Their conclusion was that acetyl-CoA is primarily made through reductive carboxylation of glutamine-derived α-ketoglutarate, suggesting potential therapeutic targets along this pathway for inhibiting hypoxic tumor growth. These studies followed previous studies of IDH reductive carboxylation flux in normal liver and cardiac cells (14–16). Here, we followed up on these studies and employed a quantitative flux model to analyze IDH flux in the same cancer cell lines. Our analysis shows that although reductive IDH flux indeed occurs in hypoxia and with mitochondrial deficiency, oxidative IDH flux persists, with net flux much less than flux in either direction.

A limitation of our analysis (as well as prior related analyses) is that it does not account for subcellular compartmentalization of most metabolites. For acetyl-CoA, we do account for the possibility of distinct labeling patterns in the mitochondria versus cytoplasm, using fatty acid labeling to infer cytosolic acetyl-CoA labeling. For other metabolites, the LC-MS approach employed here measures the isotopic labeling of the overall cellular pool, which may represent a mixture of different labeling patterns in distinct compartments (depending on the relative concentration of the metabolite in the various compartments and compartment volumes). Thus, we cannot rule out net reductive IDH flux in at least some compartments, e.g. if cytosolic and mitochondrial IDH are working in opposite directions, perhaps as a means for shuttling high energy electrons from mitochondria to cytoplasm (30, 31).

Nevertheless, a simple mechanistic explanation for the observed labeling patterns involves simultaneous oxidative and reductive IDH flux due to near equilibrium between the isocitrate oxidation and α-ketoglutarate reductive carboxylation. Such bidirectional flux can result in extensive citrate and lipid labeling via reductive carboxylation without reductive IDH being a net contributor to citrate or acetyl-CoA production. In such cases, net acetyl-CoA production may come from glucose or other carbon sources, with these influxes mixing with the larger TCA influx from glutamine via the reversible IDH reaction. The outcome is an apparent predominance of glutamine as the source of two-carbon units, despite net production coming from other sources.

More complete examination of fatty acid labeling patterns in hypoxia and pseudohypoxia reveals that glutamine labeling occurs in parallel with a rise in fatty acids that do not label from glucose or from glutamine. Such fatty acids are scavenged from media, and their assimilation into lipids decreases cellular requirements for 2-carbon unit production, thereby mitigating the need for either pyruvate dehydrogenase flux or reductive carboxylation.

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REFERENCES

1. Semenza, G. L. (2010) Oxygen homeostasis. Wiley Interdiscip. Rev. Syst. Biol. Med. 2, 336–361
2. Krebs, H. A. (1972) The Pasteur effect and the relations between respiration and fermentation. Essays Biochem. 8, 1–34
3. Kim, J. W., Tchernyshyov, I., Semenza, G. L., and Dang, C. V. (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 3, 177–185
4. Gordon, J. D., Bertout, J. A., Hu, C. J., Diehl, J. A., and Simon, M. C. (2007) HIF-2α promotes hypoxic cell proliferation by enhancing c-Myc transcriptional activity. Cancer Cell 11, 335–347
5. Wise, D. R., DeBerardinis, R. J., Mancuso, A., Sayed, N., Zhang, X. Y., Pfeiffer, H. K., Nissim, I., Daikhin, E., Yudkoff, M., McMahon, S. B., and Thompson, C. B. (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc. Natl. Acad. Sci. U.S.A. 105, 18782–18787
6. Warburg, O. (1956) On the origin of cancer cells. Science 123, 309–314
7. Zu, X. L., and Guppy, M. (2004) Cancer metabolism: facts, fantasy, and fiction. Biochem. Biophys. Res. Commun. 313, 459–465
8. Pollard, P. J., Brière, J. J., Alam, N. A., Barwell, J., Barclay, E., Wortham, N. C., Hunt, T., Mitchell, M., Olpin, S., Moat, S. J., Hargreaves, I. P., Heales, S. J., Chung, Y. L., Griffiths, J. R., Dalgleish, A., McGrath, J. A., Gleeson, M. J., Hodgson, S. V., Poulsom, R., Rustin, P., and Tomlinson, I. P. (2005) Accumulation of Krebs cycle intermediates and over-expression of HIF1α in tumours which result from germline FH and SDH mutations. Hum. Mol. Genet. 14, 2231–2239
9. Spencer, A., Corman, L., and Lowenstein, J. M. (1964) Citrate and the conversion of carbohydrate into fat. A comparison of citrate and acetate incorporation into fatty acids. Biochim. J. 93, 378–388
10. Metallo, C. M., Gameiro, P. A., Bell, E. L., Mattaini, K. R., Yang, J., Hiller, K., Jewell, C. M., Johnson, Z. R., Irvine, D. J., Guarente, L., Kelleher, I. K., Vander Heiden, M. G., Ilipoulos, O., and Stephanopoulos, G. (2012) Reductive glutamine metabolism by IDH mediates lipogenesis under hypoxia. Nature 481, 380–384
11. Mullen, A. R., Wheaton, W. W., Jin, E. S., Chen, P. H., Sullivan, L. B., Cheng, T., Yang, Y., Linehan, W. M., Chandel, N. S., and DeBerardinis, R. J. (2012) Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature 481, 385–388
12. Filipp, F. V., Scott, D. A., Ronai, Z. A., Osterman, A. L., and Smith, J. W. (2012) Reverse TCA cycle flux through isocitrate dehydrogenases 1 and 2 is required for lipogenesis in hypoxic melanoma cells. Pigment Cell Melanoma Res. 25, 375–383
13. Wise, D. R., Ward, P. S., Shay, J. E., Cross, J. R., Gruber, J. J., Sachdeva, U. M., Platt, J. M., DeMatteo, R. G., Simon, M. C., and Thompson, C. B. (2011) Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. Proc. Natl. Acad. Sci. U.S.A. 108, 19611–19616
14. Des Rosiers, B., Fernandez, C. A., David, F., and Brunengraber, H. (1994) Reversibility of the mitochondrial isocitrate dehydrogenase reaction in the perfused rat liver: evidence from isotope analysis of citric acid cycle intermediates. J. Biol. Chem. 269, 27179–27182
15. Comte, B., Vincent, G., Bouchard, B., Bendorerour, M., and Des Rosiers, C. (2002) Reverse flux through cardiac NADP+-isocitrate dehydrogenase under normoxia and ischemia. Am. J. Physiol. Heart Circ. Physiol. 283, H1505–H1514
16. Yang, L., Kasumov, T., Kombu, R. S., Zhu, S. H., Cendrowski, A. V., David, F., Anderson, V. E., Kelleher, I. K., and Brunengraber, H. (2008) Metabolic and mass isotomer analysis of liver gluconeogenesis and citric
acid cycle: II. Heterogeneity of metabolite labeling pattern. *J. Biol. Chem.* 283, 21988–21996
17. Weinman, E. O., Strisower, E. H., and Chaikoff, I. L. (1957) Conversion of fatty acids to carbohydrate; application of isotopes to this problem and role of the Krebs cycle as a synthetic pathway. *Physiol. Rev.* 37, 252–272
18. Krebs, H. A., Hems, R., Weidemann, M. J., and Speake, R. N. (1966) The fate of isotopic carbon in kidney cortex synthesizing glucose from lactate. *Biochem. J.* 101, 242–249
19. Landau, B. R., and Wahren, J. (1992) Nonproductive exchanges: the use of isotopes gone astray. *Metab. Clin. Exp.* 41, 457–459
20. Bailey, J. W., Haymond, M. W., and Miles, J. M. (1990) Validation of two-pool model for *in vivo* ketone body kinetics. *Am. J. Physiol.* 258, E850–E855
21. Schumann, W. C., Magnusson, I., Chandramouli, V., Kumaran, K., Wahren, J., and Landau, B. R. (1991) Metabolism of [2-14C]acetate and its use in assessing hepatic Krebs cycle activity and gluconeogenesis. *J. Biol. Chem.* 266, 6985–6990
22. Kosugi, K., Chandramouli, V., Kumaran, K., Schumann, W. C., and Landau, B. R. (1986) Determinants in the pathways followed by the carbons of acetone in their conversion to glucose. *J. Biol. Chem.* 261, 13179–13181
23. Kamphorst, J. J., Cross, J. R., Fan, J., de Stanchina, E., Mathew, R., White, E. P., Thompson, C. B., and Rabinowitz, J. D. (2013) Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. *Proc. Natl. Acad. Sci. U.S.A.* 110, 8882–8887
24. Lu, W., Clasquin, M. F., Melamud, E., Amador-Noguez, D., Caudy, A. A., and Rabinowitz, J. D. (2010) Metabolic analysis via reversed-phase ion-pairing liquid chromatography coupled to a stand alone orbitrap mass spectrometer. *Anal. Chem.* 82, 3212–3221
25. Lu, W., Bennett, B. D., and Rabinowitz, J. D. (2008) Analytical strategies for LC-MS-based targeted metabolomics. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 871, 236–242
26. Melamud, E., Vastag, L., and Rabinowitz, J. D. (2010) Metabolomic analysis and visualization engine for LC-MS data. *Anal. Chem.* 82, 9818–9826
27. Bennett, B. D., Yuan, J., Kimball, E. H., and Rabinowitz, J. D. (2008) Absolute quantitation of intracellular metabolite concentrations by an isotope ratio-based approach. *Nat. Protoc.* 3, 1299–1311
28. McBrian, M. A., Behbahan, I. S., Ferrari, R., Su, T., Huang, T. W., Li, K., Hong, C. S., Christofk, H. R., Vogelauer, M., Seligson, D. B., and Kurdistani, S. K. (2013) Histone acetylation regulates intracellular pH. *Mol. Cell* 49, 310–321
29. Kharroubi, A. T., Masterson, T. M., Aldaghlas, T. A., Kennedy, K. A., and Kelleher, J. K. (1992) Isotopomer spectral analysis of triglyceride fatty acid synthesis in 3T3-L1 cells. *Am. J. Physiol.* 263, E667–E675
30. Leons, J. M., Feng, X. J., Bennett, B. D., Legesse-Miller, A., Johnson, E. L., Raitman, I., Pollina, E. A., Rabitz, H. A., Rabinowitz, J. D., and Coller, H. A. (2010) Quiescent fibroblasts exhibit high metabolic activity. *PLoS Biol.* 8, e1000514
31. Ward, P. S., Patel, J., Wise, D. R., Abdel-Wahab, O., Bennett, B. D., Coller, H. A., Cross, J. R., Fantin, V. R., Hedvat, C. V., Perl, A. E., Rabinowitz, J. D., Carroll, M., Su, S. M., Sharp, K. A., Levine, R. L., and Thompson, C. B. (2010) The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* 17, 225–234