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Acetate Is a Bioenergetic Substrate for Human Glioblastoma and Brain Metastases

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SUMMARY

Glioblastomas and brain metastases are highly proliferative brain tumors with short survival times. Previously, using ^{13}C-NMR analysis of brain tumors resected from patients during infusion of ^{13}C-glucose, we demonstrated that there is robust oxidation of glucose in the citric acid cycle, yet glucose contributes less than 50% of the carbons to the acetyl-CoA pool. Here, we show that primary and metastatic mouse orthotopic brain tumors have the capacity to oxidize [1,2-^{13}C]acetate and can do so while simultaneously oxidizing [1,6-^{13}C]glucose. The tumors do not oxidize [U-^{13}C]glutamine. In vivo oxidation of [1,2-^{13}C]acetate was validated in brain tumor patients and was correlated with expression of acetyl-CoA synthetase enzyme 2, ACSS2. Together, the data demonstrate a strikingly common metabolic phenotype in diverse brain tumors that includes the ability to oxidize acetate in the citric acid cycle. This adaptation may be important for meeting the high biosynthetic and bioenergetic demands of malignant growth.

INTRODUCTION

Malignant brain tumors are among the most intractable problems in cancer. Glioblastoma (GBM), the most common and aggressive primary tumor, has a median survival of 16 months. Despite intense clinical efforts at targeting various signaling pathways, putative driver mutations, and angiogenesis mechanisms, no improvement in survival has emerged since 2005, with the addition of temozolomide to radiation as initial therapy (Cloughesy et al., 2014; Fine, 2014). Brain metastases, similarly, are aggressive tumors that affect ~200,000 patients per year in the United States (Lu-Emerson and Eichler, 2012) and usually occur late in the clinical course, often heralding end-stage disease. Treatment options are limited, and survival is measured in months (Owonikoko et al., 2014). Although GBM and brain metastases represent a broad range of cancer subtypes with distinct cellular origins and diverse genetic programs, they exhibit common metabolic characteristics that may be the result of reprogramming to enable rapid growth in the brain. Using ^{13}C-NMR, we have previously shown in patients with GBM, lung, and breast cancer brain metastases that these tumors oxidize glucose in the citric acid cycle (CAC) to produce macromolecular precursors and energy (Maher et al., 2012). The metabolic complexity of these tumors is further reflected in the identification of a "bioenergetic substrate gap," whereby a significant fraction of the acetyl-CoA pool is not derived from blood-borne glucose (Maher et al., 2012). The striking commonality of this finding among different grades of gliomas and metastatic tumors of diverse cellular origins prompted us to consider the possibility that an alternate or additional substrate(s) may serve as an important carbon source for generating CAC intermediates to support biosynthesis and bioenergetics in vivo.

Although the normal healthy adult brain relies almost exclusively on glucose as the major energy substrate, it can readily adapt to alternate fuels, including ketone bodies, short and medium chain fatty acids, and acetate (Ebert et al., 2003). Astrocytes are capable of supporting neuronal function by utilizing acetate as a metabolic substrate under conditions of limiting glucose supply, including diabetic hypoglycemia and chronic alcohol abuse (Cloughesy et al., 2104; Schurr, 2001;

Jiang et al., 2013). Because GBMs develop from the astroglial lineage, we hypothesized that these tumors retain the capacity to metabolize acetate during transformation. The most common brain metastases, in contrast, arise from organs that are not known to utilize substrates other than glucose. We speculated, that the unique brain microenvironment might drive tumors of diverse origins to utilize the same metabolic substrates to fuel aggressive growth. To test this hypothesis in vivo, we used human orthotopic tumor (HOT) mouse models of GBM and brain metastases and applied methods in intermediary metabolism for studying multiple substrates using ^{13}C-labeled nutrients (Malloy et al., 1988; Sherry et al., 1992). Coinfusion of ^{13}C-acetate and ^{13}C-glucose has been used extensively to study normal rodent brain metabolism in which differential handling of acetate and glucose by the glial and neuronal compartments can be demonstrated by ^{13}C-NMR of resected brain tissue. These methods enable direct tracing of the metabolic fate of infused substrates beyond simple uptake in the cell and therefore can be used to determine directly whether acetate can be oxidized by GBM and/or brain metastases in an orthotopic model in vivo.

Here, we report that mice harboring human GBM or brain metastases can completely oxidize acetate in the tumors. We have validated this finding in patient tumors by infusing ^{13}C-acetate in patients with GBM, breast cancer, and non-small cell lung cancer during surgical resection of their tumors and show that there is robust labeling of CAC intermediates by blood-borne ^{13}C-acetate.

In the article by Comerford et al. (2014) in this issue of *Cell*, the authors demonstrate a critical role for the nucleocytosolic acetyl-CoA synthetase enzyme, ACSS2, in hepatocellular carcinoma and broad immunoreactivity for ACSS2 in diverse human tumor types, including gliomas, breast cancer, and lung cancer. Here, we show that ACSS2 is upregulated in the HOT and primary human tumors, as well as a murine glioma model. In ACSS2 knockout mouse embryo fibroblasts (MEFs), ^{13}C-acetate fails to label CAC intermediates, and in human GBM neurospheres, stable ACSS2 knockdown leads to failure of self-renewal. These studies provide a potential mechanistic link between ACSS2 activity and in vivo acetate oxidation in tumors.

RESULTS

Glioblastomas Oxidize Acetate in the Citric Acid Cycle

The human orthotopic tumor (HOT) lines of GBM and brain metastases used in this study were each derived from an individual patient tumor, and implanted into the basal ganglia of NOD-SCID mice within 3 hr of surgical resection. All mouse experiments were approved by the Animal Resource Center, University of Texas Southwestern Medical Center (UTSW). Clinically symptomatic tumors arose within 2–4 months. The tumors, which are linked to UTSW Institutional Research Board (IRB)-approved collection of clinical information, were serially passaged and expanded in the mouse brain without adaptation to cell culture. Brain-only passaging helps ensure the preservation of the phenotypic, molecular and metabolic profiles of the human tumors and the tumor-stromal interactions, to the extent possible in an experimental system. We selected six HOT lines (UT-GBM1-6; Table S1 available online) that are representative of the most common GBM molecular profiles (Brennan et al., 2013). Each line was generated at the time of the patient's initial diagnosis prior to any treatment and was studied here in early in vivo passage. A seventh HOT line (UT-GBM7), generated at the time of repeat surgery for tumor recurrence 15 months after initial resection in the same patient from which the UT-GBM6 HOT line was derived, was chosen to compare substrate utilization in the setting of recurrence and multimodality resistance. We have validated in vivo that UT-GBM6 is temozolomide (TMZ) sensitive, whereas UT-GBM7 is TMZ resistant (Sagiyama et al., 2014).

Representative histological sections from UT-GBM1 (Figure 1A) show an expansive mass (T) comprised of densely packed tumor cells and infiltration into the brain at the leading edges. In each mouse, the contralateral hemisphere served as a matched control for substrate utilization. It is referred to as "non-tumor bearing brain" (NT; Figure 1A) rather than "normal brain" because the brain surrounding a large tumor is subjected to mass effect, an increase in reactive astrocytes and diffusible factors from the tumor and/or blood, conditions which could potentially impact brain metabolism. ^{13}C-NMR analysis of NT brain provides a valuable internal control for each mouse because the NT and T are exposed to the same circulating concentrations of ^{13}C-glucose and ^{13}C-acetate and systemic condition of the mouse. Thus, differences in labeling patterns and substrate utilization between NT and T reflect tumor-specific handling of the substrates.

[1,6-^{13}C]glucose and [1,2-^{13}C]acetate were chosen for coinfusion because oxidation of each substrate produces distinct labeling patterns in CAC intermediates, enabling a direct comparison of substrate utilization in a given tissue (Malloy et al., 1988; Taylor et al., 1996; Deelchand et al., 2009). In the schema (Figure 1B), metabolism of [1,6-^{13}C]glucose (blue circles) leads to labeling of carbon 3 in pyruvate, followed by production of acetyl-CoA labeled in position 2, which condenses with oxaloacetate (OAA), leading to labeling of carbon 4 in α-ketoglutarate (α-KG), glutamate, and glutamine during the first turn of the CAC, and in carbons 3 and 4 with subsequent turns. This labeling generates a singlet (S) and doublet 3, 4 (D34) in glutamate C4 (GLU4) and glutamine C4 (GLN4) (for example, Figure 1C). In contrast, metabolism of [1,2-^{13}C]acetate leads to labeling of both carbons (red circles) of acetyl-CoA with subsequent labeling of carbons 4 and 5 of α-KG and glutamate in the first turn of the CAC, generating a doublet 4,5 (D45) (for example, Figure 1C). In subsequent turns of the cycle, labeling in carbons 3, 4, and 5 generates a doublet of doublets (quartet, Q) (for example, Figure 2A). Thus, the ^{13}C-NMR multiplet pattern in carbon 4 of glutamate (GLU4) reflects differential labeling of the acetyl-CoA pool and provides a direct and unequivocal readout of substrate metabolism, whereby D45 and Q report acetate oxidation and S and D34 report glucose oxidation (Sherry et al., 1992). The fractional amount of each multiplet in GLU4 can be obtained by determining the area of each multiplet relative to the total spectral area of GLU4, in which the areas of S, D34, D45, and Q sum to one (Marin-Valencia et al., 2012b).

The NT brain ^{13}C-NMR spectrum (Figure 1C) shows high signal to noise and well-resolved multiplets arising from ^{13}C-^{13}C coupling. Oxidation of both glucose and acetate in NT brain is demonstrated by the presence of S and D34 from glucose and