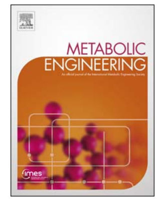
Metabolic Engineering 43 (2017) 198–207

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben



Quantitative metabolic flux analysis reveals an unconventional pathway of fatty acid synthesis in cancer cells deficient for the mitochondrial citrate transport protein

Lei Jiang[a,*,1], Adam Boufersaoui[a,f,1], Chendong Yang[a], Bookyung Ko[a], Dinesh Rakheja[d,e], Gerardo Guevara[a,g], Zeping Hu[a], Ralph J. DeBerardinis[a,b,c,**]

[a] Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA
[b] Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA
[c] McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA
[d] Departments of Pathology and Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA
[e] Department of Pathology and Laboratory Medicine, Children's Health, 1935 Medical District Drive, Dallas, TX 75235, USA
[f] Royal Stoke University Hospital, Newcastle Rd, Stoke-on-Trent, ST4 6QG, United Kingdom
[g] University of Texas at Dallas, 800 W Campbell Rd, Richardson, TX, USA

ARTICLE INFO

Keywords:
SLC25A1
Citrate transporter
2HG
IDH1
Lung cancer
Cancer metabolism

ABSTRACT

The mitochondrial citrate transport protein (CTP), encoded by *SLC25A1*, accommodates bidirectional trafficking of citrate between the mitochondria and cytosol, supporting lipid biosynthesis and redox homeostasis. Genetic CTP deficiency causes a fatal neurodevelopmental syndrome associated with the accumulation of L- and D-2-hydroxyglutaric acid, and elevated CTP expression is associated with poor prognosis in several types of cancer, emphasizing the importance of this transporter in multiple human pathologies. Here we describe the metabolic consequences of CTP deficiency in cancer cells. As expected from the phenotype of CTP-deficient humans, somatic CTP loss in cancer cells induces broad dysregulation of mitochondrial metabolism, resulting in accumulation of lactate and of the L- and D- enantiomers of 2-hydroxyglutarate (2HG) and depletion of TCA cycle intermediates. It also eliminates mitochondrial import of citrate from the cytosol. To quantify the impact of CTP deficiency on metabolic flux, cells were cultured with a set of ^{13}C-glucose and ^{13}C-glutamine tracers with resulting data integrated by metabolic flux analysis (MFA). CTP-deficient cells displayed a major restructuring of central carbon metabolism, including suppression of pyruvate dehydrogenase (PDH) and induction of glucose-dependent anaplerosis through pyruvate carboxylase (PC). We also observed an unusual lipogenic pathway in which carbon from glucose supplies mitochondrial production of alpha-ketoglutarate (AKG), which is then trafficked to the cytosol and used to supply reductive carboxylation by isocitrate dehydrogenase 1 (IDH1). The resulting citrate is cleaved to produce lipogenic acetyl-CoA, thereby completing a novel pathway of glucose-dependent reductive carboxylation. In CTP deficient cells, IDH1 inhibition suppresses lipogenesis from either glucose or glutamine, implicating IDH1 as a required component of fatty acid synthesis in states of CTP deficiency.

1. Introduction

The mitochondrial citrate transport protein (CTP, known elsewhere in the literature as the citrate carrier, CIC) mediates the transport of citrate across the inner mitochondrial membrane in electroneutral exchange with either another tricarboxylate, phosphoenoylpyruvate (PEP) or a dicarboxylate such as malate (Palmieri, 2004). CTP is encoded by the *SLC25A1* gene located on chromosome 22q11.2. *SLC25A1* expression is highest in lipogenic tissues (such as liver and renal cortex) and pancreas, reflecting key functions of mitochondrial citrate export. The first is to use cytosolic citrate as a ‘signal of plenty’, regulating glycolysis via allosteric inhibition of phosphofructokinase,

** Corresponding author at: Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA.
* Corresponding author.
E-mail addresses: lei3.jiang@utsouthwestern.edu (L. Jiang), Ralph.Deberardinis@UTSouthwestern.edu (R.J. DeBerardinis).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.ymben.2016.11.004
Received 29 July 2016; Received in revised form 13 October 2016; Accepted 7 November 2016
Available online 14 November 2016
1096-7176/ © 2016 International Metabolic Engineering Society. Published by Elsevier Inc. All rights reserved.

and fatty acid synthesis via allosteric activation of acetyl-CoA carboxylase. Citrate exported from the mitochondria in excess of the demands for oxidative metabolism also provides a source of activated acetyl units for lipogenesis and for the post-translational modification of proteins including enzymes (Xiong and Guan, 2012), transporters (Palmieri et al., 2015) and histones (Wellen et al., 2009).

In humans, biallelic germline loss of function mutations in *SLC25A1* have been linked to two distinct phenotypes. The first is a neurodevelopmental syndrome associated with combined L-/D-2-hydroxyglutaric acidaemia (Nota et al., 2013) and lactic acidosis (Prasun et al., 2015) alongside variable dysmorphic features (Smith et al., 2016). The second is an isolated early-onset congenital myasthenia (muscle weakness) in the absence of accumulated organic acids (Chaouch et al., 2014). In the latter phenotype, it appears that a moderate degree of residual CTP activity tempers the clinical severity. Haploinsufficiency of *SLC25A1* is also observed in 22q11.2 deletion syndrome (22qDS), the most common human chromosomal microdeletion, which is associated with developmental defects of the heart, face, palate, thymus and hypoparathyroid glands. Six of the 30 genes hemizygously deleted in 22qDS, including *SLC25A1*, encode mitochondrial proteins, and elevated lactate and 2HG have occasionally been observed in affected children (Napoli et al., 2015). *SLC25A1*-deficient mice are stunted and succumb to early death (Brommage et al., 2014).

Oncogenic reprogramming of metabolism in cancer cells involves many activities contributing to cellular fitness and growth, including increased rates of *de novo* fatty acid synthesis (Menendez and Lupu, 2007) and alterations in the acetylation of histones and other proteins (Taylor et al., 2014). Several lines of evidence implicate *SLC25A1* in cancer progression. High levels of *SLC25A1* expression are associated with poor prognosis in lung and estrogen receptor negative breast cancer (Kolukula et al., 2014). Breast cancer cell lines require CTP to maintain mitochondrial integrity and cell proliferation, with CTP inhibition resulting in enhanced lactate secretion and reduced oxidative phosphorylation (Catalina-Rodriguez et al., 2012). Oncogenic p53 mutants induce CTP expression and enhance the growth of xenografts derived from non-small cell lung cancer cell lines, and CTP inhibition blunts the tumorigenic effects of mutant p53 (Kolukula et al., 2014). In ovarian cancer patients, tumor *SLC25A1* mRNA levels are also associated with resistance to platinum-based chemotherapy, and blocking CTP function enhances platinum sensitivity in cultured ovarian carcinoma cells (Kolukula et al., 2014).

In this study, we sought to test whether somatic deletion of CTP in cultured cells recapitulates the phenotype of germline CTP deficiency, including alterations in lactate and 2HG metabolism. We also sought to broadly characterize the effects of CTP loss on metabolic flux in cancer cells, thereby obtaining a detailed understanding of how CTP participates in metabolic reprogramming and how compensatory metabolic activities help to sustain cell viability and growth under conditions of CTP loss.

2. Materials and methods

2.1. Materials

Culture materials were purchased from Sigma-Aldrich (St. Louis, MO). The IDH1 inhibitor GSK864 was obtained from The Structural Genomics Consortium organization (www.thesgc.org). [U-^{13}C]glucose, [U-^{13}C]glutamine, [5-^{13}C]glutamine, [3,4-^{13}C]glucose and [3-^{2}H]glucose tracers were purchased from Cambridge Isotope Laboratories (Andover, MA).

2.2. Cell lines and culture

SLC25A1-deficient H460 cells were generated using the CRISPR/Cas9 system as described (Jiang et al., 2016). H460 cells were cultured in RPMI supplemented with penicillin/streptomycin, 5% fetal bovine serum (FBS), 4 mM L-glutamine and 1 mM HEPES. Conventional tissue culture dishes were used for monolayer culture, and dishes with an Ultra-Low Attachment surface were used for spheroid culture. Identical culture medium was used for monolayer and spheroid cultures. For spheroids, 2×10^5 H460 cells were plated in a 10 cm Ultra-Low Attachment dish. The medium was changed on days 4, 6 and 7 of culture, by centrifuging at 50×*g* for 3 min, then gently resuspending in fresh medium (Jiang et al., 2016).

2.3. Western blotting

Whole cell lysates were prepared in RIPA buffer and quantified using the BCA Protein Assay (ThermoFisher Scientific). Proteins were separated on 4–20% SDS-PAGE gels, transferred to PVDF membranes, and probed with antibodies against PDH (#459400, ThermoFisher Scientific), PDH-pSer293 (AP1062, Millipore), Actin (A3853, Sigma), PC (sc-67021) and CTP (sc-86392) from Santa Cruz Biotechnology.

2.4. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR)

H460 cells were plated in growth media at 1×10^4 cells per well in XF96 microplates (Seahorse Bioscience; Billerica, MA) 18 h prior to the assay following manufacturer's recommendations for cell seeding. One hour prior to the assay, cells were washed and transferred to phenol-red free RPMI (ThermoFisher Scientific) and then incubated at 37 °C in a non-CO_2 incubator. ECAR and OCR were monitored through the Seahorse Cell Mito Stress Test, and data were normalized by protein content.

2.5. Metabolic assays and stable isotope tracing

Glucose, lactate, glutamine and glutamate were measured in culture medium using an automated electrochemical analyzer (BioProfile Basic-4 analyzer, NOVA). Stable isotope tracing experiments to determine isotopologue distributions in soluble metabolites and fatty acids were performed as described previously (Jiang et al., 2016; Yang et al., 2014).

2.6. Metabolomics

For metabolomics experiments, 3×10^6 cells were plated 24 h prior to extraction using standard culture medium. Two hours prior to extraction, the culture medium was replaced with fresh medium. Sample preparation, LC/MS and data analysis were performed as previous described (Mullen et al., 2014). Data were analyzed using a supervised partial least squares-discriminant analysis (PLS-DA) analysis, and variable importance in the projection (VIP) scores were calculated using MetaboAnalyst (Xia et al., 2015).

2.7. Measurement of 2-Hydroxyglutaric Acid (2HG)

Intracellular metabolites were extracted with 1 ml 50% methanol. Intracellular L and D enantiomers of 2HG were then measured using a LC-MS/MS based method as previous described (Rakheja et al., 2011).

2.8. Metabolic flux analysis (MFA)

Steady state metabolic fluxes were calculated by combining extracellular flux rates (glucose/glutamine utilization, lactate/glutamate secretion) and ^{13}C mass isotopologue distributions (MIDs) for lactate, citrate, glutamate, fumarate, malate and palmitate, using the isotopomer network compartmental analysis (INCA) software package (Young, 2014), which applies an elementary metabolite unit framework to efficiently simulate MIDs (Antoniewicz et al., 2007; Young et al., 2008). We developed reaction networks describing the stoichiometry and

Download English Version:

https://daneshyari.com/en/article/6452665

Download Persian Version:

https://daneshyari.com/article/6452665

Daneshyari.com