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Probing pyruvate metabolism in normal and mutant fibroblast cell lines using ^{13}C-labeled mass isotopomer analysis and mass spectrometry

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ABSTRACT

Fibroblast cell lines are frequently used to diagnose genetic mitochondrial defects in children. The effect of enzyme deficiency on overall flux rate through metabolic pathways is, however, not generally considered. We have transposed an experimental paradigm that was developed for isolated perfused organs using ^{13}C-labeled substrates and ^{13}C-isotopomer analysis to probe pyruvate mitochondrial metabolism in cultured human fibroblast cell lines with normal or genetically mutant pyruvate decarboxylation (PDC) or carboxylation (PC) activity. Cells were incubated with 1 mM [U-^{13}C]pyruvate, and the ^{13}C-molar percent enrichment (MPE) of intracellular pyruvate, citrate, malate (as a surrogate of oxaloacetate) and aspartate was assessed by mass spectrometry.

We estimated various flux ratios relevant to metabolic pathways involved in energy production, namely pyruvate formation, PDC, PC, and citrate recycling in the citric acid cycle (CAC). In all cell lines, exogenous pyruvate was predominately decarboxylated (PC/PDC ratios 0.01–0.3). PC-deficient cell lines displayed an expected negligible contribution of PC flux to oxaloacetate formation for citrate synthesis (PC/CS), which was associated with a greater contribution of PDC to acetyl-CoA formation (PDC/CS), and greater recycling of ^{13}C-labeled citrate into the CAC. In PDH-deficient cell lines, metabolic flux alterations were most apparent in cells with more than 50% reduction in enzyme activity. This led to an unexpected lower PC/CS flux ratio, while the PDC/CS flux ratio was unchanged. These data illustrate the usefulness of this approach in identifying unexpected metabolic consequences of genetic defects related to pyruvate metabolism.

Introduction

Mitochondrial cytopathies represent a heterogeneous group of multisystem disorders that preferentially affect the muscle and nervous system. They are caused either by mutations in the maternally inherited mitochondrial genome, or by nuclear DNA mutations [1]. While a number of these gene mutations have been identified and their impact on the activity of their individually encoded protein characterized, the design of treatment for patients with these mitochondrial genetic diseases would benefit from a better understanding of the metabolic and functional consequences of each of these mutations on mitochondrial and cellular energy substrate metabolism, as a whole.

Over the past decades, we have had a continuous research interest in genetic defects of mitochondrial pyruvate metabolism into the citric acid cycle (CAC)[1] [2–4]. Specifically, these include pyruvate decarboxylation (PDC) to acetyl-CoA, which is catalyzed by the pyruvate dehydrogenase complex, and anaplerotic pyruvate carboxylation (PC) to oxaloacetate (OAA) catalyzed by the pyruvate carboxylase [4,2]. While the activity of the former reaction is essential for the production of energy from carbohydrates in all cells, the latter plays a crucial role in hepatic gluconeogenesis and neurotrans-

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[1] *Abbreviation used:* CAC, citric acid cycle; DCA, dichloroacetate; GCMS, gas chromatography mass spectrometry; MPE, molar percent enrichment; MIDA, mass isotopomer distribution analysis; NMR, nuclear magnetic resonance; MIDA, mass isotopomer distribution analysis; PC, pyruvate carboxylase; PDC, pyruvate decarboxylase; PDHc, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase.

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mitter synthesis [5–7]. Genetic defects in PDC as well as PC are characterized by hyperlactacidemia with normal blood lactate-to-pyruvate ratios (⩽20) [5], which is a metabolic phenotype that resembles many respiratory chain abnormalities [3]. Hence, a definitive diagnosis of PDC deficiency is usually confirmed by measurements of enzyme activity in cultured cells or isolated tissue biopsy, followed by DNA analysis [5]. However, much remains to be learned about the metabolic consequences of genetic defects of PDC and PC on mitochondrial pyruvate metabolism.

The use of ^{13}C-labeled substrates either carbohydrates (such as glucose, pyruvate or lactate), or fatty acids, particularly uniformly labeled substrates combined with ^{13}C-isotopomer analysis by mass spectrometry (MS) and nuclear magnetic resonance (NMR) has been shown to be a very valuable approach for investigating the dynamics of mitochondrial energy substrate metabolism into the CAC [8–10]. While this approach has been successfully applied to isolated organs (liver or heart), and more recently to cultured cancer cells [11–13] to the best of our knowledge, its application to cultured mutant fibroblast cell lines, which are commonly used for genetic metabolic diagnosis, has been limited [3,14–16]. Two studies have incubated these cells with [U-$^{13}C_6$]glucose and extrapolated CAC-related metabolic fluxes using computer modeling of the isotopomeric pattern of NMR-derived intracellular ^{13}C-glutamate [14] or GCMS-derived ^{13}C-labeled amino acids and lactate from the media or proteins [15]. However, one underlying assumption of these studies is that the ^{13}C-labeling of these metabolites reflect that of the CAC intermediates, but the latter has not been directly assessed to validate the assumption. Furthermore, none of these approaches provides a precise estimate of the relative flux through anaplerotic pyruvate carboxylation, a process important for biosynthetic processes in tissues, particularly liver and brain [6,7].

The objective of this study was to develop an experimental paradigm based on measurements of the ^{13}C-labeling of CAC intermediates to assess mitochondrial pyruvate metabolism, specifically its partitioning between decarboxylation (PDC) and anaplerotic carboxylation (PC) in human fibroblast cell lines. This was achieved by transposing the experimental paradigm that was previously developed for isolated perfused organs, which is based on the use of ^{13}C-labeled substrates and ^{13}C-isotopomer analysis [9]. Specifically, fibroblast cells were incubated with 1 mM [U-^{13}C]pyruvate, followed by MS determination of the ^{13}C-labeling of CAC-

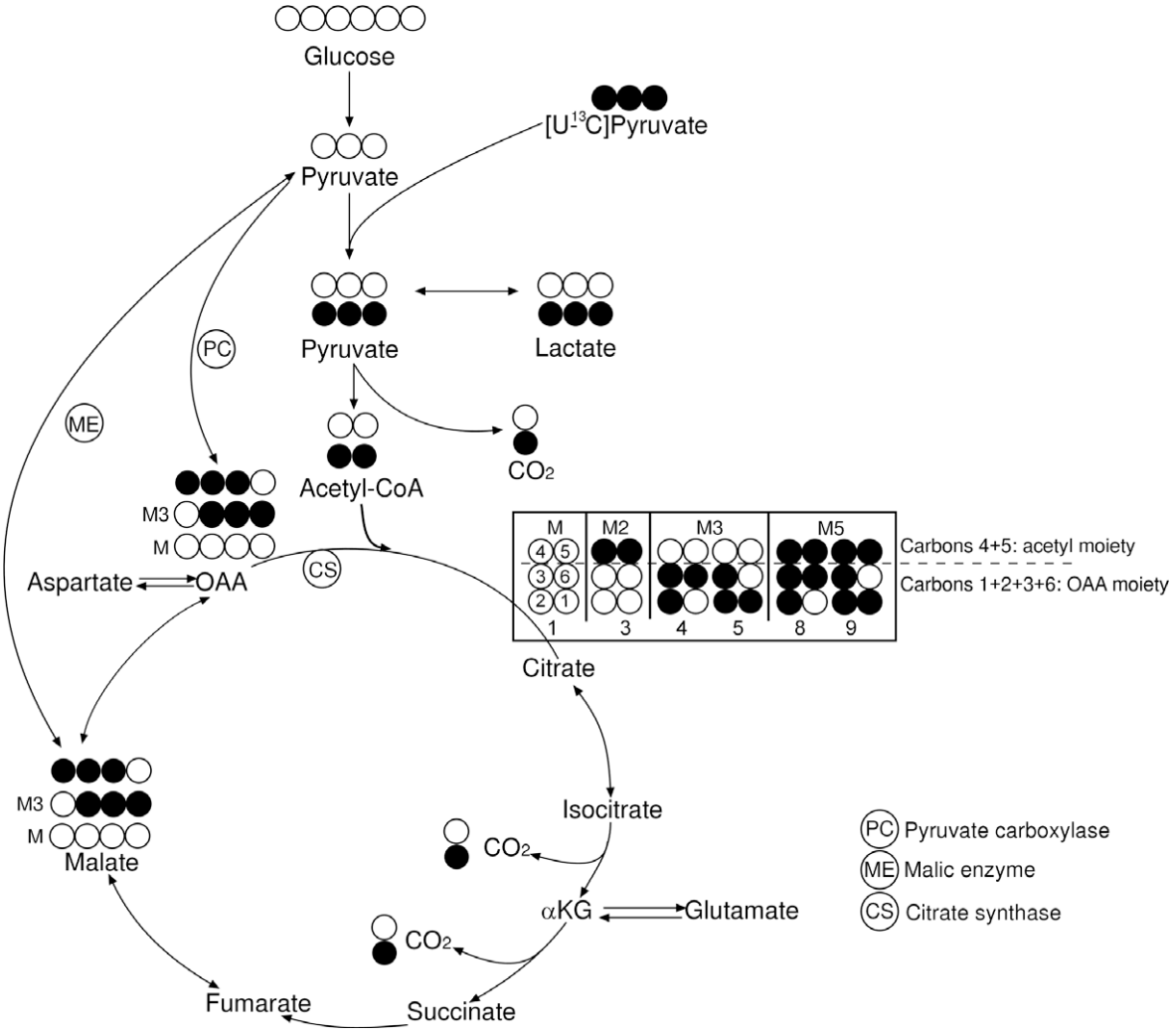

Fig. 1. Schematic demonstrating the ^{13}C-labeling of citrate with [U-^{13}C]pyruvate at the end of the first turn of the CAC cycle; with pyruvate entering the CAC via decarboxylation to acetyl-CoA and anaplerotic carboxylation to oxaloacetate and/or malate. The decarboxylation of [U-$^{13}C_3$]pyruvate generates [1,2-$^{13}C_2$]acetyl-CoA, while its carboxylation yields [1,2,3-^{13}C]OAA. Assuming complete equilibration via fumarase, [2,3,4-^{13}C]OAA is also formed in equal proportion. Through the citrate synthesis reaction, [1,2-$^{13}C_2$]acetyl-CoA (M2) will label carbons 4 + 5 of citrate (i.e. M2 isotopomers), while [1,2,3-^{13}C]OAA or [2,3,4-^{13}C]OAA (M3) will label carbons 6 + 3 + 2 and 3 + 2 + 1 of citrate (i.e. M3 isotopomers), respectively. The condensation of M2 acetyl-CoA and M3 OAA will form M4 and M5 citrate isotopomers. Upon further metabolism in the CAC, these citrate isotopomers form a mixture of M2 and M1 positional isotopomers of OAA, which will condense with unlabeled or [1,2-$^{13}C_2$]acetyl-CoA to form additional citrate isotopomers (for e.g. M1 and M3, not shown). Aspartate mass isotopomers were analyzed by LCMS/MS.