

Fluxing Through Cancer: Tracking the Fate of ^{13}C -Labeled Energy Sources Glucose and Glutamine in Cancer Cells and Mouse Tumors

Costas A. Lyssiotis,^{1,2} Susanne B. Breitkopf,^{1,2} Min Yuan,¹ Gary Bellinger,¹ John M. Asara^{1,2}

1. Beth Israel Deaconess Medical Center, Boston, MA 02215 USA

2. Harvard Medical School, Boston, MA 02115 USA

Abstract

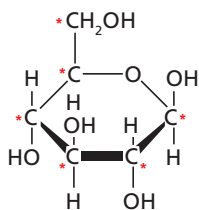
Glucose and glutamine provide the primary energy sources for cell growth and proliferation. To study metabolic reprogramming, we used D-Glucose ($\text{U-}^{13}\text{C}_6$, 99%) (CLM-1396) and L-Glutamine ($^{13}\text{C}_5$, 99%) (CLM-1822-H) to target and track the diversion of these molecules into several metabolic pathways, including glycolysis, the TCA cycle, the pentose phosphate pathway, the metabolism of amino acids and nucleotides, etc. in both cell lines and mouse tumors. We use a positive/negative ion polarity switching single column SRM experiment during a 15-minute acquisition. For *in vivo* labeling experiments, D-Glucose ($\text{U-}^{13}\text{C}_6$,

99%) or L-Glutamine ($^{13}\text{C}_5$, 99%) solutions were delivered to tumors via intraperitoneal injection (IP) or jugular delivery and compared. Metabolites were extracted from cells or tumor tissues using 80% methanol. Metabolomics were performed on a AB/SCIEX 5500 QTRAP in SRM mode using amide XBridge HILIC chromatography with Q1/Q3 transitions for both the unlabeled and ^{13}C -labeled metabolites with separate methods for glucose and glutamine.

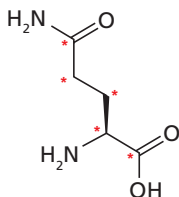
Experimental Design for Metabolic Carbon Labeling

Labeled carbon source

U^{13}C_6 -labeled glucose



U^{13}C_5 -labeled glutamine



Label carbons *in vivo*

Spike into media during cell culture

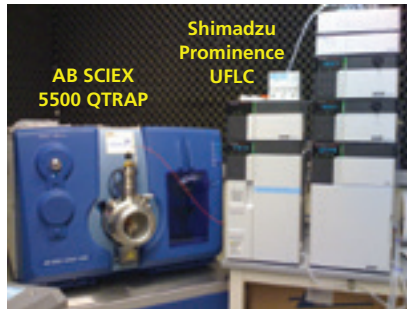


Mouse intraperitoneal (IP) injection in mouse

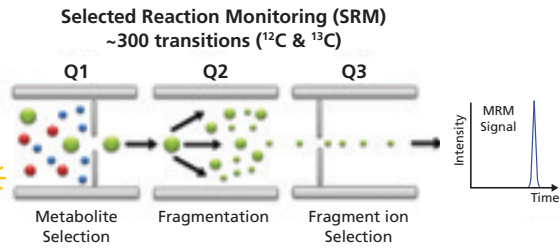


Extract metabolites for LC-MS/MS

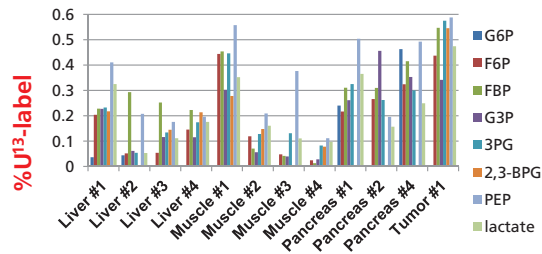
Targeted LC-MS/MS Platform for Metabolic Carbon Labeling



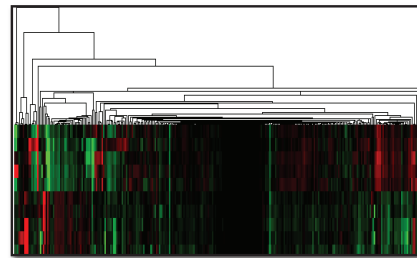
+/- switching Amide HILIC 1 column
4.6mm x 15cm
pH=9.0, NH₄⁺
400 μL / min



Trace labeled carbons through mouse organs in glucose metabolism

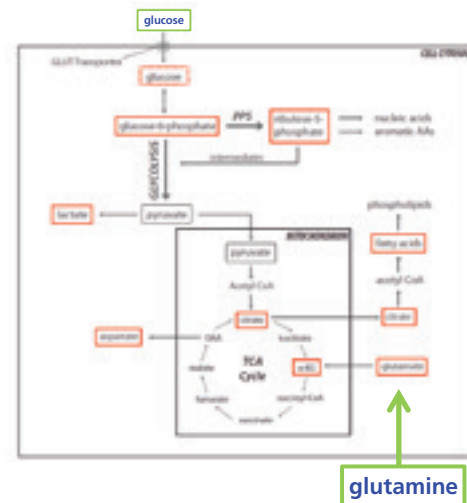
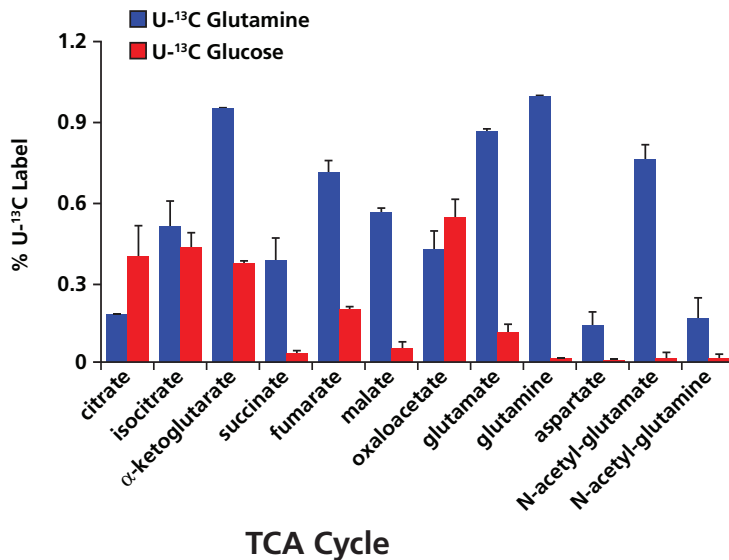


Heat map clustering of ¹³C-labeled carbons across pathways



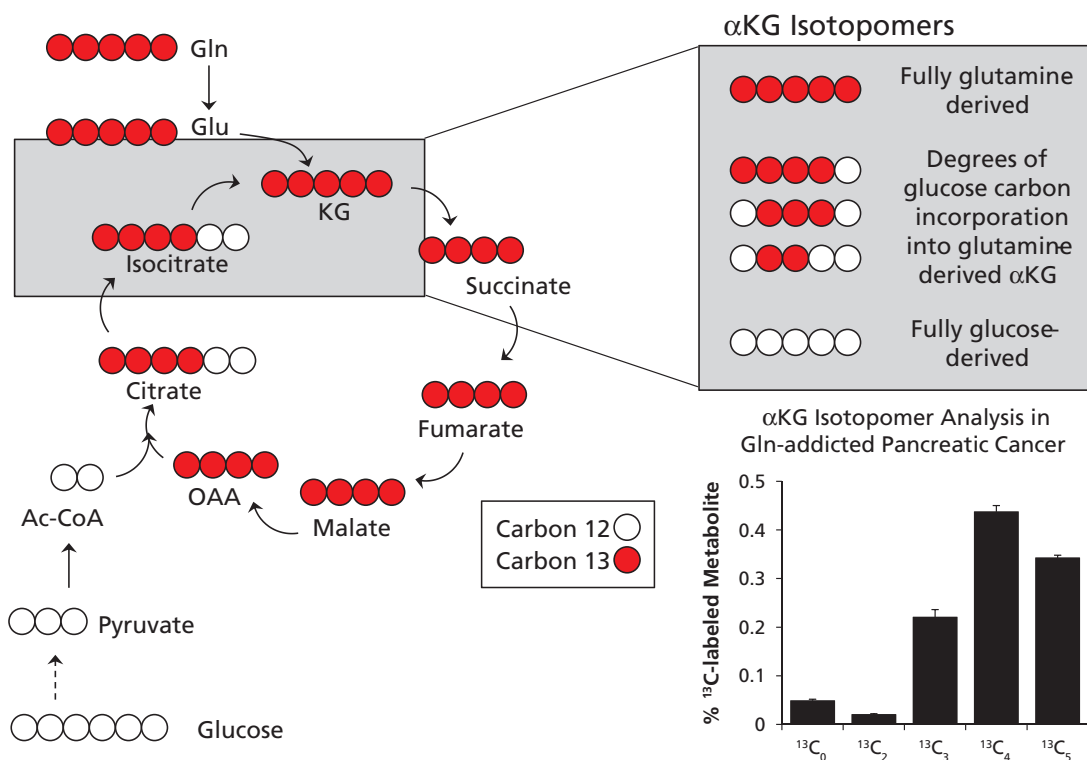
Yuan, M. et. al. 2012. *Nat Protoc*, 7, 872-81.

Glutamine Predominantly Fuels the TCA Cycle in Pancreatic Cancer



Ying, H. et. al. 2012. *Cell*, 149, 656-70.

Tracking Glutamine Metabolism by Isotope-Labeled Carbon

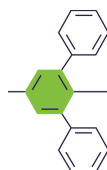


Son, J. et. al. **2013**. *Nature*, 496, 101-5.

The platform targets more than 150 labeled metabolites (>250 unlabeled metabolites). Peaks were integrated using MultiQuant software and data analyzed using in-house developed tools, as well as MetaboAnalyst, MarkerView, etc. Cell line experiments were performed in biological triplicates and assays were derived from various cancers, including multiple myeloma and pancreatic cancer that had mutations or perturbations in a number of the genes known to affect cancer metabolism. The quantitative data from *in vivo* mouse models show the specific pathways where ¹³C-labeled carbons from glucose or glutamine trace through metabolism providing valuable information regarding the defective and amplified metabolic pathways and could aid in the selection of therapeutic molecules that interfere with such pathways.

Related Products

Catalog No.	Description
CLM-1396	D-Glucose (U- ¹³ C ₆ , 99%)
CLM-1822-H	L-Glutamine (¹³ C ₅ , 99%)



BUChem BV

Orders BENELUX

Buchem B.V.
Minden 60/62
7327 AW Apeldoorn
The Netherlands

Tel +31 (0)55 543 00 40
Fax +31 (0)55 543 00 50
Email info@buchem.com
Web www.buchem.com
www.isotope.nl



Cambridge Isotope Laboratories, Inc., 3 Highwood Drive, Tewksbury, MA 01876 USA

tel: +1.978.749.8000

fax: +1.978.749.2768

1.800.322.1174 (North America)

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2/14 Supersedes all previously published literature