In Vivo Stable Isotope Labeling of ^{13}C and ^{15}N Labeled Metabolites and Lipids

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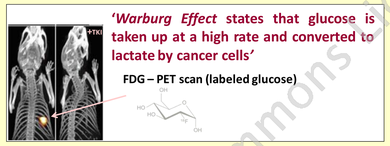
Abstract

Glucose and glutamine provide the primary energy sources for cells to grow and proliferate. Data emerging in the past decade illustrate that common genetic abnormalities in cancer converge on a program of metabolic disregulation that enhances glucose and glutamine utilization and thereby provides cancer cells with a proliferative advantage. To study metabolic reprogramming, we used 13C-labeled glucose and 13C-labeled glutamine to target and track the diversion of these molecules into several metabolic pathways, including glycolysis, the TCA cycle, the pentose phosphate pathway, lipid synthesis, the metabolism of amino acids and nucleotides and various others in both cell lines and mouse tumors. We approach with either a single column positive/negative switching SRM experiment during a 15 min acquisition for polar metabolites or a pos/neg switching DDA and or MS1-AIF experiment for lipids and fatty acids or metabolites.

Methods

For *in vivo* labeling experiments, $U^{13}C_6$-labeled glucose, $U^{13}C_5$-glutamine or $U^{15}N_1$-glutamine solutions were added to glucose/glutamine-free media for cell lines or delivered to tumors via intraperitoneal injection (IP). Metabolites were extracted from cells or tumor tissues using 80% cold methanol and lipids were extracted using methyl tert butyl ether (MTBE). Metabolomics were performed on a AB/SCIEX 5500 QTRAP in SRM mode (>270 unlabeled metabolites, 150 labeled metabolites) using amide XBridge HILIC or XBridge C_{18} chromatography (Waters). Lipidomics were performed on a Thermo QExactive Plus (QE+) in DDA and MS1-DIA modes. Both platforms use positive/negative switching from a single 15-20 min LC-MS/MS experiment. For 5500 data, peaks were integrated using MultiQuant software and analyzed using MetaboAnalyst, MarkerView and in-house developed tools. QE+ data were analyzed using SIEVE, LipidSearch and TraceFinder software.

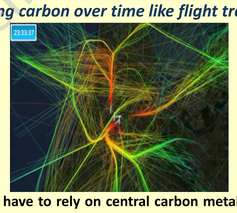
Tumors and highly metabolic cells love glucose



'Warburg Effect states that glucose is taken up at a high rate and converted to lactate by cancer cells'

FDG – PET scan (labeled glucose)

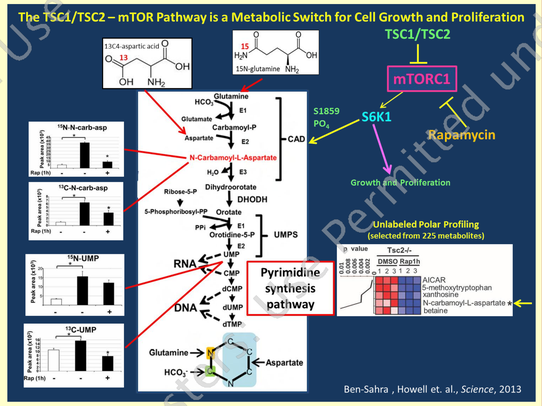
Tracing carbon over time like flight tracking



Regardless of the genetic defects that cancer cells possess, they have to rely on central carbon metabolism for growth and survival. FDG PET is a great example that cancers take up glucose at high rates. Mass spectrometry can be used to trace the carbon atoms from glucose and glutamine into metabolites and lipids.

Results: *Targeting* $^{13}C/^{15}N$ Labeled Metabolites

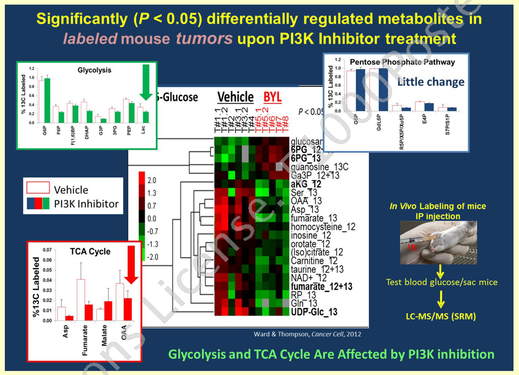
Tracking ^{13}C through Glycolysis, TCA Cycle and Pentose Phosphate Pathway



13C and 15N labeled glutamine and aspartic acid were used to trace carbons through the first 3 steps of *de novo* pyrimidine synthesis. Phosphopeptide data correlated with metabolomics data proving that mTOR controls this process.

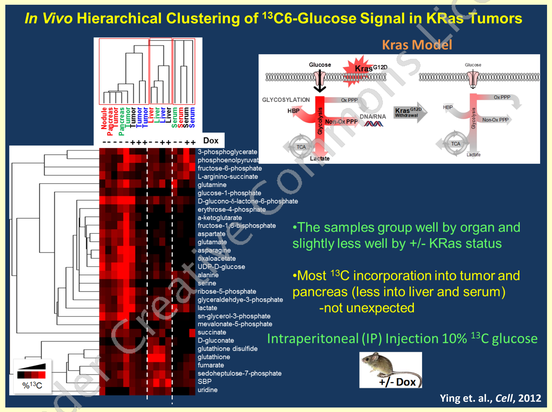
Results: *In Vivo* ^{13}C Labeling of Mice

In vivo ^{13}C glucose labeling BRCA1 Mice Treated with PI3K Inhibitor



^{13}C glucose and/or glutamine (10% solutions) can be injected into the mouse using several methods including intraperitoneal injection, jugular vein bolus and tail vein. With 60% labeling efficiency, significant metabolic changes can be observed.

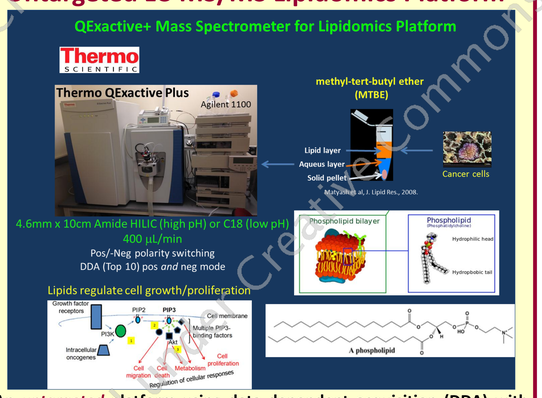
In Vivo ^{13}C Glucose Incorporation into KRas Mouse Tumors



Polar metabolomics from injecting ^{13}C labeled glucose into mice and harvesting the tumors and other organs was successful in distinguishing metabolic changes between KRas activation states (+/- Dox). Glucose shunts to the non-oxidative pentose phosphate pathway in KRas G12D tumors.

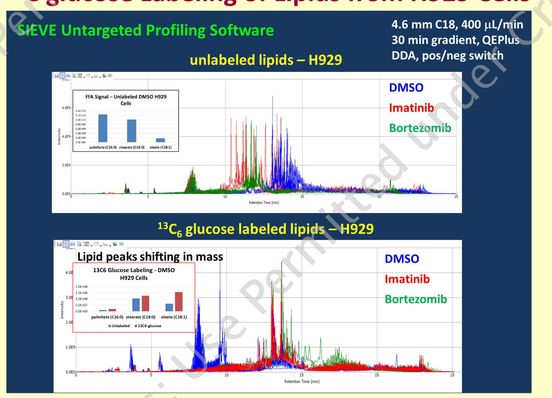
Results: *Untargeted* ^{13}C Labeled Lipidomics & Metabolomics

Untargeted LC-MS/MS Lipidomics Platform



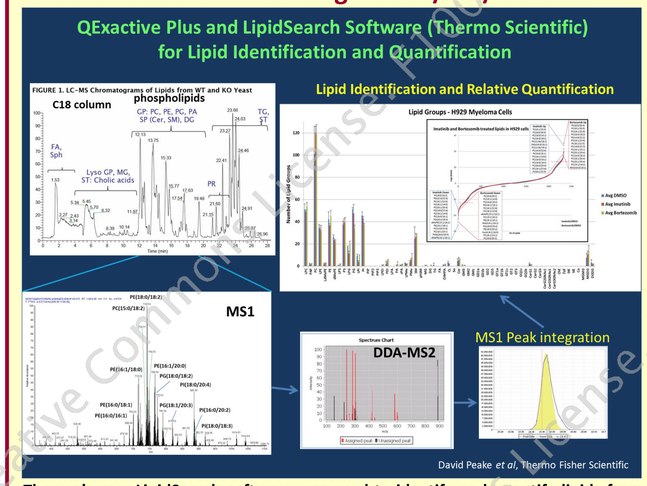
An *untargeted* platform using data dependent acquisition (DDA) with pos/neg switching was developed using the QExactive Plus mass spectrometer for *lipidomics* profiling.

^{13}C glucose Labeling of Lipids from H929 Cells



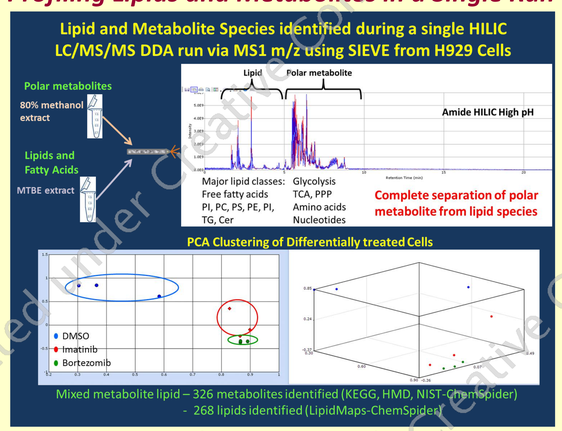
^{13}C-labeled glucose was added to H929 cells and unbiased LC-MS/MS profiling using the QE+ showed that carbon atoms were incorporated into several lipid species based on *m/z* shifting after lipid extractions.

Use of LipidSearch software for unbiased Lipid Identification from Untargeted LC/MS/MS data



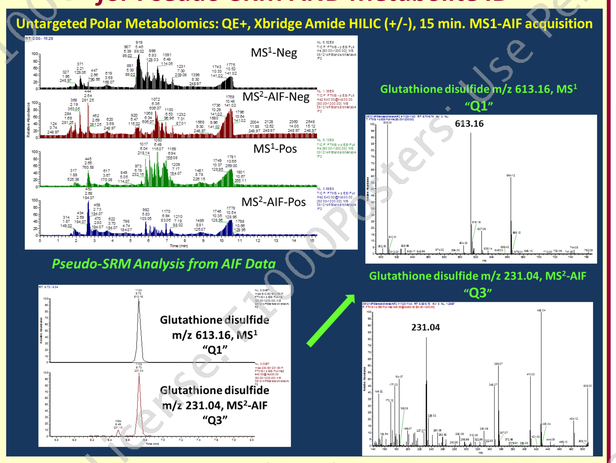
Thermo's new LipidSearch software was used to identify and quantify lipids from H929 myeloma cells from our *untargeted* QExactive Plus DDA-pos/neg switch platform over several drug conditions.

Profiling Lipids and Metabolites in a Single Run



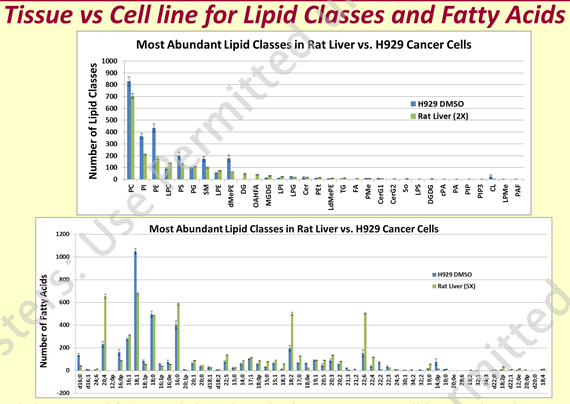
A DDA platform with pos/neg switching from m/z 80-1200 using the QE+ was implemented for both *lipidomics* and *metabolomics* profiling in the *same* 20 min. LC-MS/MS run using high pH HILIC chromatography since metabolites and lipids elute in different chromatographic regions.

QExactive Plus in All Ion Fragmentation (AIF) mode for Pseudo-SRM AND Metabolite ID



Precursor-All Ion Fragmentation (MS1-AIF) acquisitions on the QE+ using pos/neg switching to replace (or complement) traditional SRM acquisitions for polar metabolites and some lipids. It also has the ability to re-probe datasets for peaks of interest (by precursor mass and fragment ions)

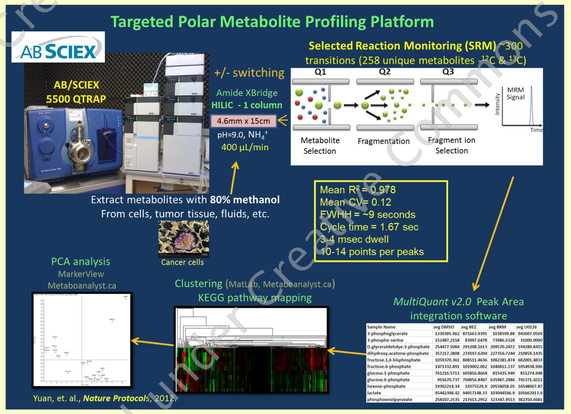
Tissue vs Cell line for Lipid Classes and Fatty Acids



Lipid classes and fatty acid chain lengths from a cancer cell line and rat liver tissue were compared using DDA pos/neg profiling on the QE+ and LipidSearch software.

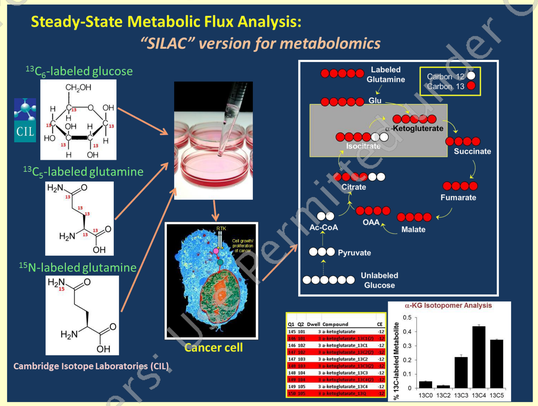
Results: *Targeted Metabolomics Platform via SRM*

Targeted Polar Metabolite Profiling Platform



SRM LC-MS/MS platform for polar metabolic profiling using a single 15 min. HILIC column run with positive/negative polarity switching at 3 msec dwell times with no chromatographic scheduling.

^{13}C and ^{15}N Labeling of Cell Lines and Targeted Tracing



Metabolic pathways in established cell lines are labeled with $^{13}C_6$-glucose, $^{13}C_5$-glutamine or $^{15}N_1$-glutamine (Cambridge Isotope Labs) to 11 mM (glucose) or 2 mM (glutamine) and maintained for 16-24 hr.

Conclusions

- Targeted MS can be used successfully track the fate of ^{13}C or ^{15}N labeled molecules *In Vivo*
- Tracking the activated or altered metabolic pathways in cancers is important for recognizing cancer cell types that have a growth survival benefit
- Understanding these altered metabolic pathways is important for determining how we can intervene to block these pathways
- Untargeted DDA and AIF on a QExactive Plus MS are effective methods for detecting and quantifying lipids and metabolites in addition to SRM methods on the 5500 QTRAP MS for rigorous quantitation