FluxSearch: A Strategy for $^{13}$C/$^{15}$N Metabolic and Lipid Flux Analysis from Untargeted High Resolution LC-MS/MS

He Huang$^{1,2}$, Min Yuan$^1$, Gerburg M. Wulf$^{1,2}$, John M. Asara$^{1,2}$

$^1$Beth Israel Deaconess Medical Center, Boston, MA USA  $^2$Harvard Medical School, Boston, MA USA

Abstract

$^{13}$C metabolite flux is typically performed in a targeted manner on known metabolic pathway intermediates, mostly involving central carbon metabolism. Untargeted and unbiased flux analysis is difficult because there are multiple steps including metabolite identification, tracking of carbon atoms into both precursors and fragments, requirements for high resolution and mass accuracy, etc. We developed a platform to combine untargeted LC-MS/MS with stable isotopic tracing (such as $^{13}$C glucose, $^{13}$C palmitate or $^{15}$N glutamine) labeling in order to identify and perform flux analysis on any molecule that can be confidently identified using both high mass accuracy precursor and fragment ion information. This FluxSearch method can perform metabolic and/or lipid flux analysis on any molecule that can be identified by untargeted LC-MS/MS.

Methods

Various cancer cell lines were labeled with $^{13}$C-glucose and their metabolites, lipids and polypeptides were extracted with MTBE/methanol from various lipid layers. Extracts were run using high resolution LC-MS/MS (QExactive HR/HRMS) in data dependent acquisition (DDA) mode with polarity switching with online HPLC chromatography (metabolites) or C$_{18}$ chromatography (lips and peptides). Elements software with NIST MSMS spectral libraries were used to identify unique metabolites from the middle layer. Lipids from the top layer were identified using LipidSearch software. Polypeptides were identified from the middle layer and proteins were depleted from trypsin from the bottom pellet. Identification and peak lists produced using enviPick and then imported into house developed FluxSearch software to identify all isotopomers of $^{13}$C incorporation up to a maximum of ten $^{13}$C carbon atoms for metabolites and forty carbon atoms for lipids.

Results

FluxSearch can profile metabolic and lipidic flux from cell lines in-house developed FluxSearch software to interpret $^{13}$C isotopic incorporation; 50 carbon incorporation for metabolites, and 40 carbon incorporation for lipids.

Conclusions

- A novel strategy for calculating $^{13}$C/$^{15}$N flux information for any molecule identified in a biological system by untargeted LC-MS/MS
- A triple negative breast cancer cell line treated with PI3K/AKT inhibitors at different time-courses were successfully analyzed by the FluxSearch platform to reveal the connections between protein signaling and metabolism and lipid flux regulations.

References