



METHODOLOGY

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Decoding the dynamics of cellular metabolism and the action of 3-bromopyruvate and 2-deoxyglucose using pulsed stable isotope-resolved metabolomics

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Abstract

Background: Cellular metabolism is highly dynamic and continuously adjusts to the physiological program of the cell. The regulation of metabolism appears at all biological levels: (post-) transcriptional, (post-) translational, and allosteric. This regulatory information is expressed in the metabolome, but in a complex manner. To decode such complex information, new methods are needed in order to facilitate dynamic metabolic characterization at high resolution.

Results: Here, we describe pulsed stable isotope-resolved metabolomics (pSIRM) as a tool for the dynamic metabolic characterization of cellular metabolism. We have adapted gas chromatography-coupled mass spectrometric methods for metabolomic profiling and stable isotope-resolved metabolomics. In addition, we have improved robustness and reproducibility and implemented a strategy for the absolute quantification of metabolites.

Conclusions: By way of examples, we have applied this methodology to characterize central carbon metabolism of a panel of cancer cell lines and to determine the mode of metabolic inhibition of glycolytic inhibitors in times ranging from minutes to hours. Using pSIRM, we observed that 2-deoxyglucose is a metabolic inhibitor, but does not directly act on the glycolytic cascade.

Keywords: Stable isotope labeling, GC-MS, Cancer metabolism, Glycolysis, Metabolomics, 3-Bromopyruvate, 2-Deoxyglucose

Background

Central metabolism is highly flexible and continuously adjusts to the physiological program of the cell, organ, and organism. In a healthy state, cellular metabolism is tightly regulated to guarantee physiological function and the efficient usage of available resources. However, the underlying mechanisms leading to metabolic dysfunction are often poorly understood. To gain deeper insights into the dynamics of metabolism, techniques enabling a quantitative and time-resolved analysis of the metabolome are needed to allow a detailed real-time view of carbon

routing through central carbon metabolism (CCM): glycolysis, pentose phosphate pathway (PPP), tricarboxylic acid cycle (TCA) cycle, and amino acid metabolism.

Metabolic flux is an expression of the activity of a network of enzymatic reactions. This network is influenced by several factors: the expression levels of the catalytic enzymes and the relative concentrations of substrates and products. In addition, allosteric regulation and post-translational modifications, including phosphorylation, ubiquitylation and, most recently recognized, acetylation, control metabolic activity. Metabolic flux cannot be inferred from transcript or protein levels of constituent enzymes, but rather requires direct measurement. Several techniques for metabolic flux analysis (MFA) have been established previously. Flux balance analysis (FBA) and stoichiometric MFA rely on

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