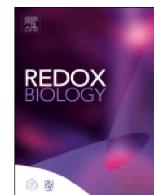
Redox Biology 16 (2018) 381–387

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox



Research paper

Sorting cells alters their redox state and cellular metabolome

Elizabeth M. Llufrio[a], Lingjue Wang[a], Fuad J. Naser[a], Gary J. Patti[a,b,*]

[a] Department of Chemistry, Washington University, St. Louis, MO 63130, United States
[b] Department of Medicine, Washington University, School of Medicine, St. Louis, MO 63110, United States

ABSTRACT

A growing appreciation of the metabolic artifacts of cell culture has generated heightened enthusiasm for performing metabolomics on populations of cells purified from tissues and biofluids. Fluorescence activated cell sorting, or FACS, is a widely used experimental approach to purify specific cell types from complex heterogeneous samples. Here we show that FACS introduces oxidative stress and alters the metabolic state of cells. Compared to unsorted controls, astrocytes subjected to FACS prior to metabolomic analysis showed altered ratios of GSSG to GSH, NADPH to $NADP^+$, and NAD^+ to NADH. Additionally, a 50% increase in reactive oxygen species was observed in astrocytes subjected to FACS relative to unsorted controls. At a more comprehensive scale, nearly half of the metabolomic features that we profiled by liquid chromatography/mass spectrometry were changed by at least 1.5-fold in intensity due to cell sorting. Some specific metabolites identified to have significantly altered levels as a result of cell sorting included glycogen, nucleosides, amino acids, central carbon metabolites, and acylcarnitines. Although the addition of fetal bovine serum to the cell-sorting buffer decreased oxidative stress and attenuated changes in metabolite concentrations, fetal bovine serum did not preserve the metabolic state of the cells during FACS. We conclude that, irrespective of buffer components and data-normalization strategies we examined, metabolomic results from sorted cells do not accurately reflect physiological conditions prior to sorting.

1. Introduction

Liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/mass spectrometry (GC/MS) are the most widely used experimental platforms for performing metabolomics [1]. Historically, these technologies have been primarily applied to two types of samples: (i) cells grown in standard monoculture, or (ii) tissues and biofluids harvested from animals and patients. Cell monoculture has some attractive benefits, such as being cost effective and high throughput. Most important to the current work, cell monoculture avoids the challenge of having to resolve metabolites from more than one cell type. As a consequence, the metabolism of cultured cells can be rapidly quenched and their metabolites extracted for profiling without a cell-purification step [2]. Metabolomic analysis of tissues and biofluids by LC/MS or GC/MS, in contrast, is complicated by the presence of multiple cell types. Without a cell-purification step, signal intensities in metabolomic data represent the average concentration of a metabolite from all cell types in the tissue or biofluid and are difficult to interpret in the context of metabolic regulation. Thus, historically, cells in monoculture have been primarily used to study metabolic regulation by LC/MS or GC/MS, whereas whole tissues and biofluids have more frequently been used to screen for biomarkers of disease [3–5].

A potential complication of studying metabolic regulation in cell culture is that cells are not in their naturally occurring environment, which can introduce non-physiological artifacts in metabolism [6,7]. Standard cell-culture media, for instance, contains ~10-fold less fatty acids compared to healthy human serum. Proliferating cells have a high demand for fatty acids to support the formation of new membranes. In standard cell-culture media, proliferating cells mostly synthesize fatty acids *de novo* from glucose [8]. When proliferating cells are cultured in media containing physiological levels of fatty acids, however, they prefer to uptake the fatty acids rather than synthesize them. Although media formulations are emerging that better reflect the composition of human plasma, nutrient availability may not be the only source of metabolic artifacts in cell culture [9]. Despite having access to glutamine, for example, some tumors show minimal utilization of glutamine in vivo. Yet, cell lines derived from these same tumors rely heavily on glutamine in cell culture [10].

With increasing evidence that the metabolism of cells in culture differs from the metabolism of cells in an animal or a patient, there has been heightened enthusiasm to study metabolic regulation in tissues and biofluids with metabolomics. The challenge remains of how to

* Corresponding author.
E-mail address: gjpattij@wustl.edu (G.J. Patti).

https://doi.org/10.1016/j.redox.2018.03.004
Received 5 February 2018; Received in revised form 3 March 2018; Accepted 6 March 2018
Available online 09 March 2018
2213-2317/ © 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

resolve the metabolites of specific cell types within the samples during LC/MS and GC/MS profiling. An experimental strategy that is commonly employed to purify populations of cells from complex samples is fluorescence-activated cell sorting or FACS [11]. One potential workflow is to isolate specific types of cells from complex samples by FACS and subsequently quench their metabolism prior to extracting metabolites for mass spectrometry analysis [12]. While it is provocative to imagine stopping metabolism by enzyme inactivation prior to cell sorting, conventional methods for quenching metabolism are not compatible with FACS. It is therefore important to note that FACS can take up to several hours, depending on experimental conditions, sample type, and number of replicates. Many metabolites turnover on a much faster timescale [13]. By way of illustration, the total pool of ATP can turnover six times per minute in heart tissue [14]. During FACS, cells are transferred to buffers with limited nutrient availability and then subjected to changes in temperature as well as pressure. Here we sought to assess the extent that such environmental perturbations during FACS reprogram cellular metabolism, which has important implications for the physiological relevance of metabolomic data collected from sorted cells.

In this study, we found that subjecting astrocytes to FACS led to oxidative stress and an altered redox state as supported by significant changes in the ratios of NADPH to $NADP^+$ and NAD^+ to NADH. In mammalian cells, NAD(H) and NADP(H) are utilized by hundreds of metabolic reactions that span various biochemical functions. Many of these reactions are regulated by the ratios of NADPH to $NADP^+$ and NAD^+ to NADH [15]. Hence, it may not be surprising that we also found changes in the concentrations of metabolites involved in many major metabolic pathways as a result of FACS. Our work indicates that metabolomic data from sorted cells do not accurately reflect the native metabolism of cells prior to FACS.

2. Materials and methods

2.1. Tissue culture

Cells were grown in high-glucose Dulbecco's Modified Eagle Media (DMEM) (4.5 g/L glucose) containing 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin at 37 °C with 5% CO_2.

2.2. Morphology and viability assay

DI TNC1 astrocytes, plated with the same original seeding density, were left in 1 mL of phosphate buffered saline (PBS) with or without 1% dialyzed FBS (dFBS) at 4 °C for 4 h. Bright field images were taken by using a BioTek Cytation™ 5 Cell Imaging Multi-Mode Reader. Viability and cell number were assessed by using trypan blue and a Nexcelom Cellometer Auto 1000.

2.3. FACS

Cells were resuspended to form a single cell suspension in 1 mL of PBS alone, 1% dFBS in PBS, or 1% bovine serum albumin (BSA) in PBS. The cell suspension was filtered and then sorted by using FACS on a BD FACSAria™ II (nozzle size 85 μM, plate voltage 5000 V, sheath pressure 45 psi, flow rate 1.0, optical path/laser used = 488 nm). Cells were selected with side scatter and forward scatter to collect live, single cells [16,17]. The population was gated based on the relative size and complexity of the cells using Forward Scatter (FSC) and Side Scatter (SSC) parameters. Doublets were excluded with FSC-Width and SSC-Width (Fig. S1).

2.4. Reactive Oxygen Species (ROS) detection

ROS were detected with the DCFDA/H_2DCFDA - Cellular Reactive Oxygen Species Detection Assay Kit from Abcam (Cat. No. ab113851) according to the manufacturer's instructions. In brief, 2',7'-dichlorofluorescin diacetate (DCFDA) was added to the cells. Oxidation of DCFDA by ROS was monitored by the formation for 2',7'-dichlorofluoroscein (DCF). Fluorescence of DCF was measured (Ex/Em = 485/535 nm).

2.5. Glycogen detection

Glycogen was measured by using a Glycogen Assay Kit from Abcam (Cat. No. 65620) according to the manufacturer's instructions (OxiRed probe, Ex/Em = 535/587 nm).

2.6. Assessing NADPH/$NADP^+$

The $NADP^+$/NADPH Quantitation Colorimetric Kit from BioVision (Cat. No. K347) was used according to the manufacturer's instructions. In brief, fluorescence of the NADPH Developer was measured (OD = 450 nm), two hours after the addition of the NADP Cycling Mix.

2.7. Hydrogen peroxide treatment

For the H_2O_2 metabolomic experiments, cells were treated with three mL of PBS +/− 1% dFBS and 200 μM H_2O_2 at 4 °C for 4 h before quenching metabolism with methanol (MeOH).

2.8. LC/MS-based metabolomics

Cells in the rapidly quenched condition had their metabolism quenched quickly with 500 μL MeOH/ 6×10^5 cells after removal from cell-culture plates. Cells in the sorted condition were first subjected to FACS before being quenched with 500 μL MeOH/ 6×10^5 cells. Cells in the delayed-quench condition were left on the benchtop in PBS alone or with 1% dFBS for four hours before being quenched with 500 μL MeOH/ 6×10^5 cells. After extraction, samples were analyzed with hydrophilic interaction liquid chromatography (HILIC) or reversed-phase liquid chromatography (RPLC) coupled to mass spectrometry in negative or positive ionization mode, respectively. All raw data files were converted into mzXML files using msconvert [18]. Data analysis was performed by using either Xcalibur Qual Browser or a combination of in-house software packages implemented in R, which we have described in detail previously [19–21].

2.9. Metabolite extraction

Cell pellets were dried on a SpeedVac and subsequently lyophilized. Metabolites were isolated from lyophilized cell pellets by using methanol/acetonitrile/water (2:2:1), adjusted to maintain a ratio of 0.5 mL of solvent per 6×10^5 cells or 1 mL/mg. Following the previously described protocol [22], extracts were dried with a SpeedVac and then reconstituted in 50 μL/6×10^5 cells or 100 μL/mg of acetonitrile/water (1:1) and placed in 4 °C for 1 h. Samples were centrifuged at 14 kRPM and 4 °C for 10 min. Supernatant was transferred to LC/MS vials for analysis.

A more detailed version of materials and methods can be found in the Supplemental information.

3. Results and discussion

To evaluate the effects of FACS on astrocytes, we subjected cells grown in monoculture to three different experimental conditions, which we refer to as rapidly quenched, sorted, or delayed quench. The rapidly quenched condition served as a control, where the metabolism of cultured cells was quickly stopped prior to any analysis. In the sorted condition, cells were subjected to FACS before having their metabolism quenched for subsequent analysis. Lastly, in the delayed-quench condition, cells were left on the benchtop for the same amount of time

Download English Version:

https://daneshyari.com/en/article/8286631

Download Persian Version:

https://daneshyari.com/article/8286631

Daneshyari.com