A Comprehensive Workflow for Routine, Automated, Metabolite + Lipid Analysis of Mammalian Cells

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Metabolomics 2020

#74

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1. Introduction

We developed an automatable workflow that harnesses a novel room temperature cell lysis and quenching method and, from the same sample, extracts and separates polar metabolites and lipids using solid phase extraction (SPE). To reduce hands-on time, increase throughput, and increase robustness of the workflow, the majority of workflow steps were automated on a liquid handling platform. This automated workflow was utilized to analyze the metabolic perturbations in K562 leukemia cells following treatment with methotrexate (MTX), which halts cellular proliferation.

Technological Advancements Provide a Room Temperature Dual Metabolite + Lipid Cell Sample Preparation Workflow

The new dual metabolite + lipid cell sample prep workflow is easily automated due to a room temperature quenching step and solid phase separation of polar metabolites and lipids.

Dual Metabolite + Lipid Sample Prep Workflow Applied to a Model System

Application of the dual metabolite + lipid workflow to investigation of MTX treatment of K562 cells led to new biological insights.

2. Results and Discussion - Lipids

Dual Metabolite + Lipid Workflow Provides Similar Lipid Extraction Profiles to a Liquid-Liquid Extraction Workflow

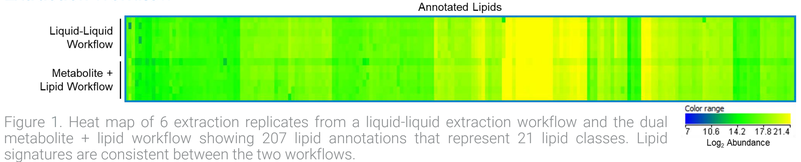

Figure 1. Heat map of 6 extraction replicates from a liquid-liquid extraction workflow and the dual metabolite + lipid workflow showing 207 lipid annotations that represent 21 lipid classes. Lipid signatures are consistent between the two workflows.

Untargeted Lipidomics Analysis finds a New Result

An untargeted metabolomics study[1] of MTX pharmacological activity in a cell model found decreases and increases in lipid classes and specific lipid species. Our comprehensive cell workflow additionally found an increase in acylcarnitines upon MTX treatment, a finding not previously reported[1].

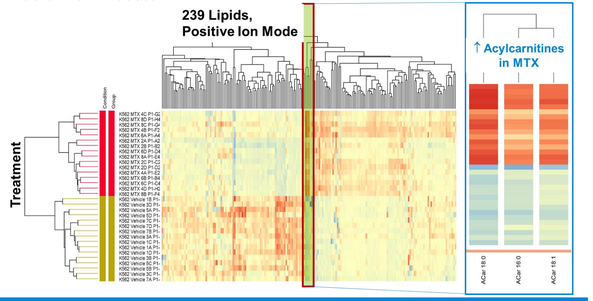

Figure 2. Heat map showing unsupervised clustering of lipids found in vehicle- and MTX- treated samples using the dual sample prep workflow and Lipid Annotator, Profinder, and MPP analysis.

Methotrexate Alters Lipid Abundances for Select Lipid Classes

Lipid class analysis of cells treated with MTX shows both increases and decreases in abundance for different lipid classes. Of note, the class decrease in phosphatidylinositols (PIs) is dominated by decreases in short-chain PIs.

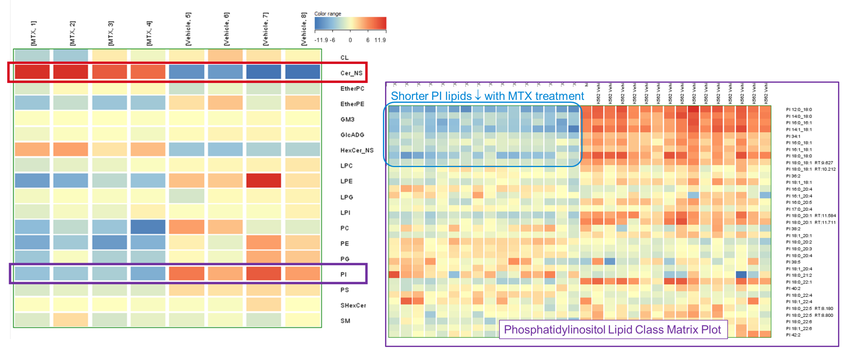

Figure 3. Methotrexate-induced lipidomic signature in K562 leukemia cells using the comprehensive dual metabolite + lipid cell sample prep and data analysis workflow. As previously shown by Funk et al.[1], ceramides (Cer_NS) increase with MTX treatment and phosphatidylinositols (PI) decrease with MTX treatment, particularly shorter PIs.

3. Results and Discussion – Polar Metabolites

Dual Metabolite + Lipid Workflow Provides Excellent Polar Metabolite Recoveries when Compared to a Liquid Extraction Workflow

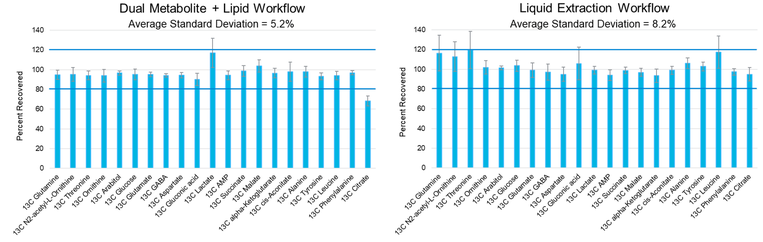

Figure 4. Recoveries from pre- and post-spiked ^{13}C-labeled metabolites

Methotrexate Alters Polar Metabolite Abundances

A recent targeted metabolomics approach[2] found MTX treatment led to accumulation of the purine metabolite intermediates AICA riboside (AICAr) / AICAR resulting in activation of AMPK. This led to increased bioenergetic capacity and decreased one-carbon metabolism and cellular proliferation. The AICAr / AICAR findings were confirmed in this study.

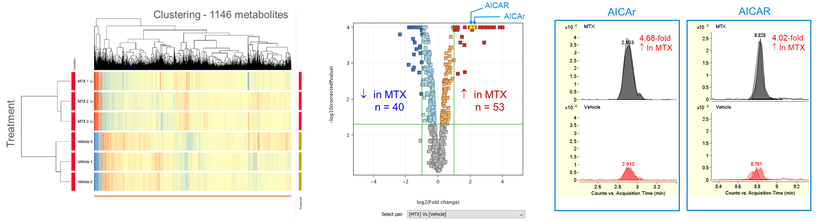

Figure 5. Methotrexate-induced polar metabolite signature in K562 leukemia cells (left), volcano plot indicating metabolites that increase or decrease significantly upon MTX treatment (middle), and AICAr / AICAR peaks (right) in vehicle and MTX-treated metabolite extracts. Note: due to the pandemic, a historical MS[1] dataset from K562 cells prepared using a liquid extraction workflow was utilized.

4. Conclusions

We describe a new room temperature, automated cell sample prep workflow that provides effective room temperature quenching of metabolism and enables extraction and separation of metabolites and lipids from the same sample using solid phase extraction. We demonstrated excellent polar metabolite recoveries and lipid signatures comparable to a liquid-liquid extraction method. This sample prep workflow, coupled with Lipid Annotator, Profinder, and Mass Profiler Professional data analysis packages, provides a comprehensive sample prep and data analysis workflow for analysis of metabolomic and lipidomic perturbations in cells. Using this workflow for an analysis of the impact of MTX on K562 cells revealed similar changes in metabolomic and lipidomic profiles as in prior studies and uncovered a previously unreported increase in acylcarnitines. This method development and validation lays the groundwork for routine, automated, intra-sample multi-omics analysis of mammalian cell samples.

References

[1]Funk, R.S. et al. Metabolomic Profiling to Identify Molecular Biomarkers of Cellular Response to Methotrexate In Vitro. Clin Transl Sci. 2020 January; 13(1): 137-146.

[2]Papadopoli, D.J. et al. Methotrexate elicits pro-respiratory and anti-growth effects by promoting AMPK signaling. Scientific Reports. 2020 May 12; 10(1): 7838.