An Improved Lipid Profiling Workflow Demonstrates Disrupted Lipogenesis Induced with Drug Treatment in Leukemia Cells

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Metabolomics 2019 Conference

POSTER # 492

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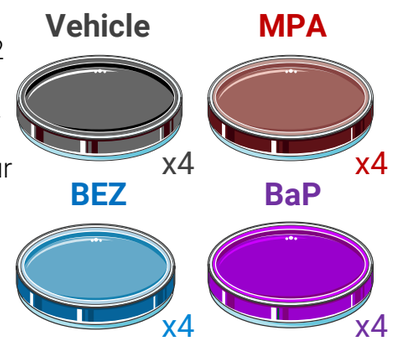
Introduction

While shotgun lipidomics has advanced the field of lipid analysis, it suffers from limitations that have led to a shift towards chromatographic-based lipid profiling approaches using HPLC coupled to HRMS. To improve these workflows, we demonstrate a novel software tool that enables product-ion spectral matching against an in-silico generated database to annotate iterative-mode MS/MS spectra. The tool takes special care not to over-annotate lipid entities and quickly generates an accurate-mass retention time (AMRT) database in an automated fashion. The resulting database annotates MS1 lipid profiling data for downstream differential analysis and incorporates new lipid-specific visualization tools. We applied this novel workflow to study lipidome alterations of an acute-myeloid-leukemia cell line in response to a drug treatment combination.

Experimental

Sample Preparation



Acute-myeloid-leukemia K562 cells were cultured in supplemented RPMI medium. Six-well plates were seeded with $2.4x10^5$ cells/mL and four different treatments were applied: 0.5 mM bezafibrate (BEZ), 5 mM medroxyprogesterone acetate (MPA), BaP (combination of 0.5 mM BEZ and 5 mM MPA), or vehicle control (1:1 ethanol:DMSO). Four replicate wells were prepared for each treatment. After incubation for 24 hours, cells were pelleted by centrifugation, washed with PBS (1 mL, 4˚C), re-pelleted, and the pellets were flash-frozen and stored at -80°C. Lipids were extracted with a modified Folch biphasic extraction procedure.

Data Acquisition

LC/MS methods were followed as detailed in Agilent Application Note 5994-0775[1]. Briefly, lipids extracts were analyzed with a 19-minute reverse-phase based LC-MS/(MS) method on an Agilent 6546 LC/Q-TOF mass spectrometer.

Data Analysis

A targeted lipidomics profiling workflow was employed as depicted in Figure 1.

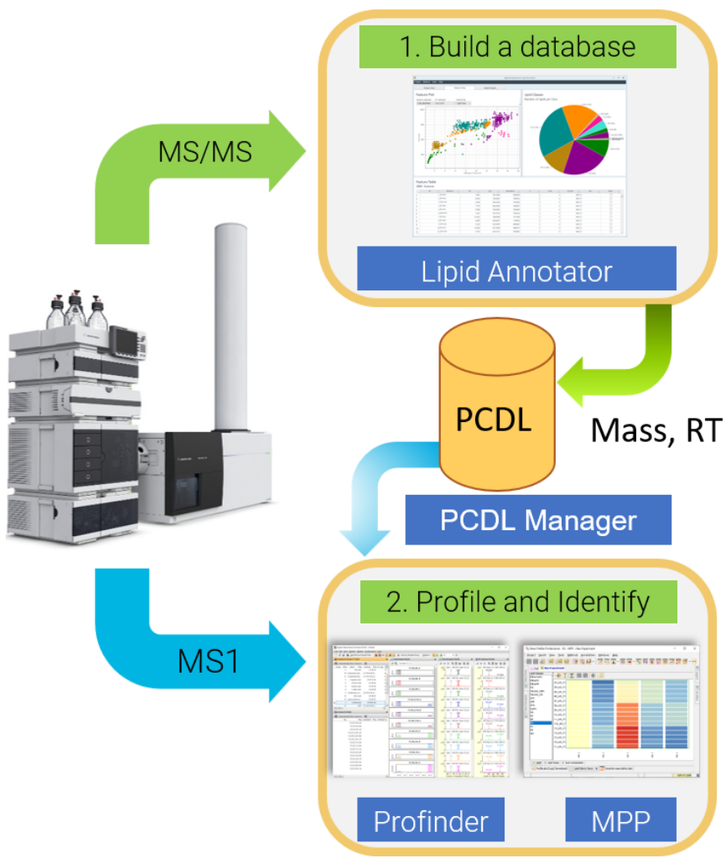


Figure 1. Targeted Lipidomics Workflow. The dark blue boxes indicate Agilent MassHunter software.

Results and Discussion

Database Generation

With Lipid Annotator software, a total of 440 features were annotated from five positive-ion Iterative MS/MS datafiles (Fig 2), and 688 features were annotated from five negative-ion datafiles. Results were used to automatically generate a custom Personal Compound Database and Library (PCDL) containing accurate masses, spectra and retention times.

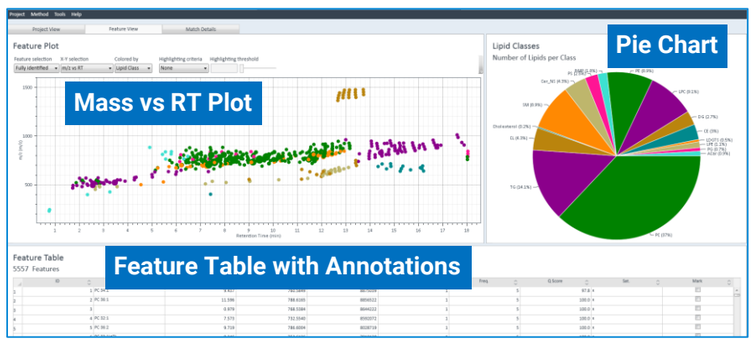


Figure 2. View of Agilent MassHunter Lipid Annotator software results from a batch of five positive-ion Iterative MS/MS datafiles acquired on a pooled K562 cellular lipid extract. Iterative MS/MS data was previously shown to improve lipid annotation coverage.[1]

Lipid Profiling Demonstrates Disrupted Lipogenesis

The PCDL was used as the formula source for Batch Targeted Feature Extraction in Profinder on the 16 MS1 datafiles. Results were imported into Mass Profiler Professional (MPP) for statistical analysis. The resulting Principal component analysis (PCA) showed clear effects of the different treatments (Fig 3). In agreement with a previous report[2], BEZ contributed most to the combination drug (BaP) effect.

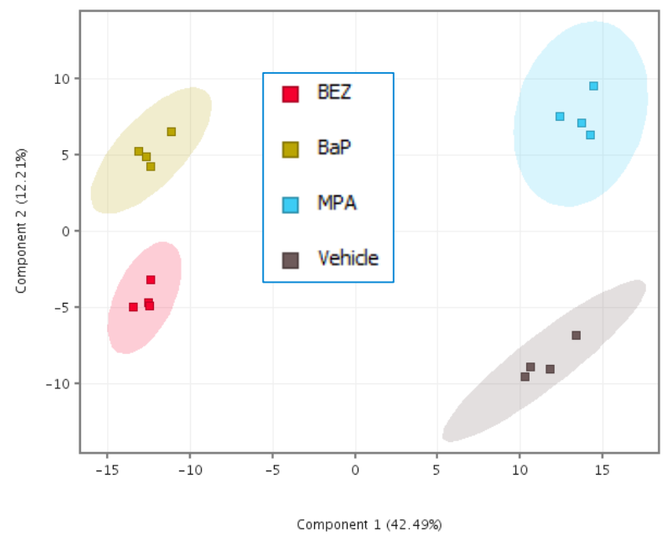
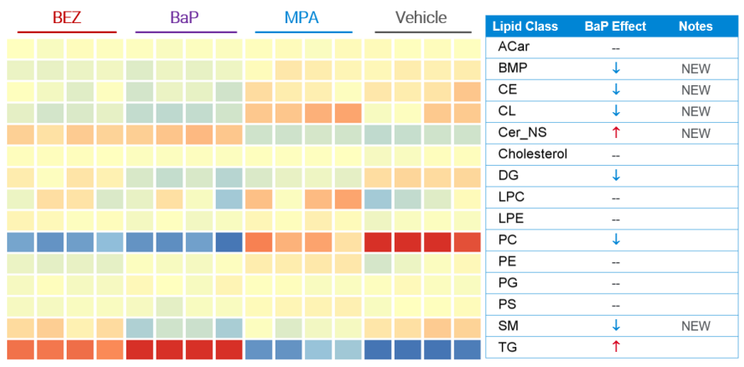


Figure 3. MPP PCA results for 341 lipid features from the positive ion dataset.

Differences in lipid class profiles were observed with drug treatment (Fig 4). Specifically, increased TAG and decreased DAG levels with BaP treatment agreed with a previous report[2], while the workflow also newly identified differences in BMP, CE, CL, Cer_NS, and SM levels.



Lipid Class	BaP Effect	Notes
ACar	--	
BMP	↓	NEW
CE	↓	NEW
CL	↓	NEW
Cer_NS	↑	NEW
Cholesterol	--	
DG	↓	
LPC	--	
LPE	--	
PC	↓	
PE	--	
PG	--	
PS	--	
SM	↓	NEW
TG	↑	

Figure 4. MPP lipid matrix (heatmap) of total normalized lipid class abundances across drug treatments. The table summarizes the observed effect of BaP treatment (↑ = increase, ↓ = decrease), and whether the effect is a new finding not previously reported.

Results and Discussion

A heatmap (not shown) of 113 phosphatidylcholine (PC) feature abundances revealed systematic trends. Inspection of two of the features showed a decrease in PCs with saturated chains and an increase in PCs with polyunsaturated chains (Fig 5).

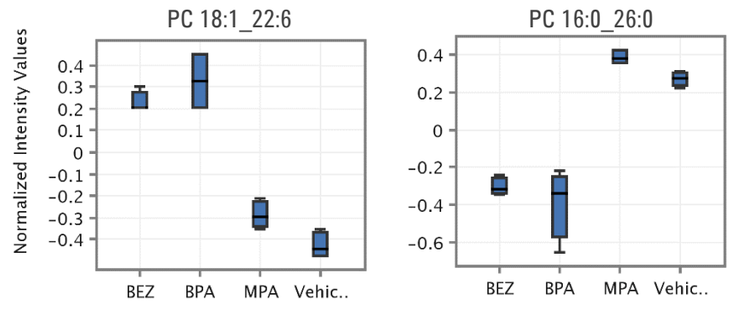


Figure 5. Boxplot comparison of two annotated PC features across drug treatments.

The LC/MS approach enabled chromatographic separation of lipid isomers with the same exact mass. Many isomers displayed differential responses to drug treatment (Fig 6). In some cases, the MS/MS spectra provided further structural information as to the nature of the isomers.

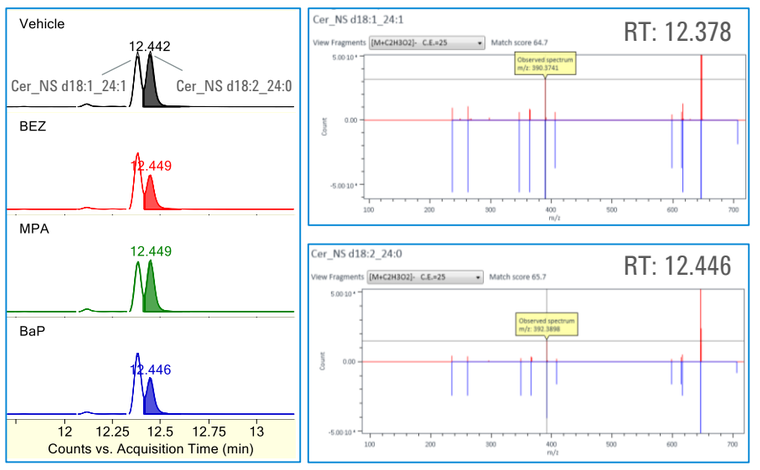


Figure 6. Profinder chromatograms for a pair of ceramide isomers (sum composition 42:2, left panel), and head-to-tail plots from Lipid Annotator (right) provided evidence for Cer_NS d18:1_24:1 and Cer_NS d18:2_24:0.

Conclusions

A novel lipidomics workflow was applied to study the effects of drug treatment on AML cells

- Results supported a previous report including a decrease in DAG, an increase in TAG, and an enrichment of PCs with polyunsaturated fatty acids with BaP treatment.
- New results demonstrated differential levels of Cer_NS, CL, SM, CE, and BMP lipid classes, including lipid isomers.

Taken together the results demonstrate that the workflow achieved a more comprehensive lipid annotation and more in-depth lipid profiling than previously published[2].

References

[1]Sartain, M, et al. Improved Coverage of the Plasma Lipidome Using Iterative MS/MS Data Acquisition Combined with Lipid Annotator Software and 6546 LC/Q-TOF. Agilent Application Note 5994-0775EN, 2019

[2]Southam, A.D. et al. Drug Redeployment to Kill Leukemia and Lymphoma Cells by Disrupting SCD1-Mediated Synthesis of Monounsaturated Fatty Acids. Cancer Res. 2015 June; 75(12): 2530-40

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