Untargeted metabolomics of Tagless Lyso-IP

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Abstract

Lysosomal biology is increasingly implicated in neurodegenerative diseases and health. It has traditionally been difficult to profile the metabolomic homeostasis of the lysosome in disease states. To overcome this challenge, we have developed the Tagless Lyso-IP method to rapidly prepare lysosome enriched samples from human peripheral blood. This protocol details the processing and untargeted analysis of polar metabolites derived using the Tagless Lyso-IP method.

Materials

Reagents

Optima LC/MS water (Fisher, cat. no. W6-4)

Optima LC/MS acetonitrile (Fisher, cat. no. A955-4)

Ammonium carbonate

Ammonium hydroxide

EASYIC™

Isotopically labeled amino acids (Cambridge Isotope Laboratories, cat. no. MSK-A2-S)

Equipment

ID-X Orbitrap Tribrid Mass Spectrometer (Thermo Fisher Scientific) with an electrospray ionization (ESI) probe

SeQuant® ZIC®-pHILIC 150 x 2.1 mm column (Millipore Sigma 1504600001)

20 x 2.1 mm guard (Millipore Sigma 1504380001)

DynaMag™ Spin Magnet. (Thermofisher scientific. Catalog #12320D)

Microcentrifuge with thermostat (VWR Micro Star 17R. S/N 42209232. REF# 521-1647)

Eppendorf ThermoMixer® C, Eppendorf, #EP02095

Thermotop, Eppendorf, #EP5308000003

Method

This method is following successful isolation of lysosomes the Tagless Lyso-IP method as described in: protocols.io xxxxxx (Tagless Lyso-IP).

Note: The for the steps following the immunoprecipitation of lysosomes (Steps 28-32) the wash buffer used is ice cold KPBS without protease and phosphatase inhibitors.

Processing of polar metabolite samples

1. Resuspend the lysosomes attached to the magnetic beads and the pelleted whole cell samples in 50 µL of 80% MeOH (v/v) with isotopically labelled amino acids and vortex briefly.
2. Resuspend your whole cell pellets in 225 µL of 80% MeOH (v/v) with isotopically labelled amino acids and vortex briefly.
3. Incubate at 4 °C for 10 min.
4. Place your Lyso-IP samples (Step 1) on a tube magnet for 30 s.
5. Transfer the supernatant into a fresh 1.5 mL Eppendorf tube.
6. Centrifuge both the Lyso-IP and the whole cell samples (Steps 2 and 5) at 13 000 x g, 4 °C for 10 min.
7. Transfer the supernatant to fresh 1.5 mL Eppendorf tubes and store at -80 °C.
8. On the day of LC/MS measurement, vortex samples at 4 °C for 10 min.
9. Centrifuge the samples at 13 000 x g, 4 °C for 10 min.
10. Transfer the supernatant to autosampler vials.

LC/MS metabolomics settings

11. Set an ID-X tribrid mass spectrometer (Thermo Fisher Scientific) with an electrospray ionization (ESI) probe, for initial polar metabolite profiling.
12. Prepare a SeQuant® ZIC®-pHILIC 150 x 2.1 mm column (Millipore Sigma 1504600001) coupled with a 20 x 2.1 mm (Millipore Sigma 1504380001) guard, to carry out hydrophilic interaction chromatography (HILIC) for metabolite separation prior to mass spectrometry. Use EASYICTM for internal calibration.
13. For HILIC metabolite separation, use 20 millimolar (mM) ammonium carbonate and 0.1 % (v/v) ammonium hydroxide dissolved in 100 % (v/v) LC/MS grade water for Buffer A, and 100 % (v/v) LC/MS grade acetonitrile for Buffer B.
14. Run the chromatographic gradient at a flow rate of 0.150 mL/min. Operate the mass spectrometer in full-scan, polarity-switching mode at m/z 70-1000, with Orbitrap resolution set at 120,000, RF lens at 40%, AGC target at 1x106, and maximum injection time at 80 ms. Set positive ion voltage to 3000 V, negative ion voltage to 2500 V, ion transfer tube temperature to 275 °C, and vaporizer temperature to 350 °C. Set sheath gas flow to 40 units, auxiliary gas flow to 15 units, and sweep gas flow to 1 unit.
15. For unbiased differential analysis, extract ion chromatograms using Compound Discoverer (Thermo Fisher Scientific) with a mass tolerance of 5 ppm. Rigorously quantify metabolite abundance using TraceFinder (Thermo Fisher Scientific) in conjunction with an in-house library of known metabolite standards (MSMLS, Sigma-Aldrich).
16. Compound Discoverer (Thermo Fisher Scientific) was used for initial unbiased differential analysis. In addition to online databases,we also included a local library with both masslist and mzVault spectral archives. Mass tolerance for untargeted discovery, 10 ppm; minimum and maximum precursor mass, 0-5,000 Da; retention time limit, 0-20 min; Peak filter signal to noise ratio, 1.5; retention time alignment maximum shift, 0.5 min; minimum peak intensity, 10,000; compound detection signal to noise ratio, 3. Isotope

and adduct settings were kept at default values. Gap filling and background filtering were performed by default settings. Area normalization was performed by constant median after blank exclusion. Compound annotation priority: #1, MassList Search; #2, mzVault Search; #3, mzCloud Search; #4, Predicted Compositions; #5, Chemspider Search; #6, Metabolika Search.

17. The MassList Search was customized with 5 ppm mass tolerance and 1 minute retention time tolerance. The mzVault Search was customized with 10 ppm precursor and fragment mass tolerance and 1 minute retention time tolerance. The mzCloud Search was customized with 10 ppm precursor and fragment mass tolerance. The other searches were performed with default parameters specified in the default workflow "Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mzLogic" provided by Compound Discoverer.
18. Raw features were further filtered by the following algorithm: 1) MS2 fragmentation spectra were obtained, and 2) at least 1 annotation match in the mzVault, mzCloud or Chemspider Search. To further improve the rigor of our discovery workflow, we performed additional manual filtering based on the following criteria: 1), features with retention time earlier than 3 minutes on this HILIC column, which are nonpolar and should be quantified by a C18 column, were removed, 2) features with predicted compositions containing chemical elements rarely found in human metabolome (e.g. certain halogens) were removed, and 3) features enriched in the Golgi from only one independent experiment were removed.
19. Rigorous quantification of metabolite abundance was performed by TraceFinder (Thermo Fisher Scientific) in conjunction with an in-house library of known metabolite standards (MSMLS, Sigma-Aldrich). Isotopically labelled amino acids were used as internal standards. Mass tolerance for extracting ion chromatograms, 5 ppm.
    a. Note: For LC wellness: make sure to transfer both WC and Golgi-IP samples from the original harvesting tubes to another NEW SET OF TUBES. When taking the supernatant from the Golgi-IP sample, USE A MAGNET TO PREVENT DRAWING UP BEADS.