

Untargeted and targeted polar metabolomic profiling

The Weill Cornell Medicine Meyer Cancer Center Proteomics & Metabolomics Core Facility performed hydrophilic interaction liquid chromatography-mass spectrometry (LC-MS) for relative quantification of polar metabolite profiles. Metabolites were measured on a Q Exactive Orbitrap mass spectrometer, coupled to a Vanquish UPLC system by an Ion Max ion source with a HESI II probe (Thermo Scientific). A Sequant ZIC-pHILIC column (2.1 mm i.d. × 150 mm, particle size of 5 µm, Millipore Sigma) was used for separation. The MS data was processed using XCalibur 4.1 (Thermo Scientific) to obtain the metabolite signal intensity for relative quantitation. Targeted identification was available for 205 metabolites based on an in-house library established using known chemical standards. Identification required exact mass (within 5ppm) and standard retention times. For untargeted metabolomics, metabolites were identified by mass matching of the MS signal to metabolites in the HMDB database. If multiple metabolites in the database were matched to a certain MS signal, all matched metabolites were grouped into a single identification, and ordered based on the number of references included in the HMDB database (high to low).

Lipidomics

Chromatographic separation was performed on a Vanquish UHPLC system with a Cadenza CD-C18 3 µm packing column (Imtakt, 2.1 mm id x 150 mm) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Scientific) via an Ion Max ion source with a HESI II probe (Thermo Scientific). The mobile phase consisted of buffer A: 60% acetonitrile, 40% water, 10 mM ammonium formate with 0.1% formic acid and buffer B: 90% isopropanol, 10% acetonitrile, 10 mM ammonium formate with 0.1% formic acid. The LC gradient was as follows: 0–1.5 min, 32% buffer B; 1.5-4 min, 32-45% buffer B; 4-5 min, 45-52% buffer B; 5-8 min, 52-58% buffer B; 8-11 min, 58-66% buffer B; 11-14 min, 66-70% buffer B; 14-18 min, 70-75% buffer B; 21-25 min, isocratic 97% buffer B, 25-25.1 min 97-32% buffer B; followed by 5 min of re-equilibration of the column before the next run. The flow rate was 200 µl/min. A data-dependent mass spectrometric acquisition method was used for lipid identification. In this method, each MS survey scan was followed by up to 10 MS/MS scans performed on the most abundant ions. Data was acquired in positive and negative mode in separate runs. The following electrospray parameters were used: spray voltage 3.0 kV, heated capillary temperature 350 °C, HESI probe temperature 350 °C, sheath gas, 35 units; auxiliary gas 10 units. For MS scans: resolution, 70,000 (at m/z 200); automatic gain control target, 3e6; maximum injection time, 200 ms; scan range, 250-1800 m/z. For MS/MS scans: resolution, 17,500 (at 200 m/z); automatic gain control target, 1e5 ions; maximum injection time, 75 ms; isolation window, 1 m/z; NCE, stepped 20,30 and 40. The LC-

MS results were processed using MS-DIAL software (version 4.9) for lipid identification and relative quantitation.

Free Fatty Acid Analyses

Chromatographic separation was performed on a Vanquish UHPLC system (Thermo Scientific) with a Cadenza CD-C18 3 μm packing column (Imtakt, 2.1 mm id x 150 mm) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Scientific) via an Ion Max ion source with a HESI II probe (Thermo Scientific). The mobile phase consisted of buffer A: 5 mM ammonium acetate in water (LC/MS grade, Thermo Scientific) and buffer B: 5 mM ammonium acetate, 85% isopropanol (LC/MS grade, Thermo Scientific), 10% acetonitrile (LC/MS grade, Thermo Scientific), and 5% water. The LC gradient was as follows: 0–1.5 min, 50% buffer B; 1.5–3 min, 50–90% buffer B; 3–5.5 min, 90–95% buffer B; 5.5–10 min, 95% buffer B, followed by 5 min of re-equilibration of the column before the next run. The flow rate was 150 $\mu\text{L}/\text{min}$. MS data was acquired in negative mode. The following electrospray parameters were used: spray voltage 3.0 kV, heated capillary temperature 350 $^{\circ}\text{C}$, HESI probe temperature 350 $^{\circ}\text{C}$, sheath gas, 35 units; auxiliary gas 10 units. For MS scans: mass scan range, 140–1000 m/z; resolution, 70,000 (at m/z 200); automatic gain control target, $1\text{e}6$; maximum injection time, 50 ms. MS data files were processed using XCalibur (version 4.1, Thermo Scientific). Identification of free fatty acids was based on accurate masses within 5 ppm and standard retention times from compound standards. Relative quantitation was performed based on MS signal intensities.