ESI 3: Methods

Omics Analyses

S1.1 Extraction of metabolites and lipids

Deep frozen cells were harvested by adding 400 µL 80% methanol (pre-cooled on dry ice) into each well. Cells were scraped from each well on dry ice and were transferred into a 1.8 mL glass vial which was pre-filled with 640 µL pre-cooled chloroform and 416 µL H2O. 400 µL 80% methanol (pre-cooled on dry ice) was used to wash each well and the wash solution was transferred into the same glass vial. After adjusting the ratio of methanol:chloroform:water (v/v/v) to 1:1:0.9, each glass vial was vortexed for 30 s twice, at 30 s intervals. The glass vials were then cooled on dry ice for 10 mins before centrifugation for 10 mins at 3000-g at -9 °C, which separated the mixture into two phases (upper polar phase and lower non-polar phase). 300 µL aliquots of the polar phase were transferred into clean 1.5mL Eppendorf tubes and then dried in a speed vac concentrator (Thermo Savant, Holbrook, NY) for 4 hr. 300 µL aliquots of the non-polar phase were transferred into clean 1.8 mL glass vials and dried under a stream of nitrogen for 5 mins. All dried samples were then frozen at -80°C until analysis.

S1.2 Direct infusion mass spectrometry (DIMS)

The DIMS analysis method used was similar to the one reported previously (Southam et al. 2007; Zhang et al. 2016). The dried polar or non-polar extracts were re-suspended in 80 µL 80:20 (v/v) methanol:water (HPLC grade) with 0.25% formic acid (for positive ion mode analysis of polar extracts) or 80 µL 2:1 methanol:chloroform with 5 mM ammonium acetate (for negative ion mode analysis of lipids). After centrifugation at 22000g, 4 °C for 10 min, 10 µL supernatant of each sample was loaded into each of four well of a 384-well plate and then analysed (in quadruplicate) using direct infusion mass spectrometry (Q Exactive, Thermo Fisher Scientific, Germany) in positive ion mode (for polar metabolomics) or negative ion mode (for lipidomics), utilising a Triversa nanoelectrospray ion source (Advion Biosciences, Ithaca, NY, USA).

S1.3 Metabolomics Data Processing

Mass spectra were recorded using the selected ion monitoring (SIM) stitching approach from m/z 50-620 (for polar metabolomics) or from m/z 50-1020 (for lipidomics) and then processed using custom-written Matlab scripts as previously reported (Kirwan et al. 2014; Southam et al. 2016). In brief, only mass spectral peaks with a signal-to-noise ratio exceeding 3.5 were retained. For each sample the combination of three of the four technical replicates that has the smallest relative standard deviation (RSD) was selected, which were aligned with a 2ppm mass range and filtered into a single peak list (with only those peaks present in ≥ 2 of the 3 spectra retained). Each filtered peak list (one per sample) was then further aligned with a 2ppm mass

range, and filtered to retain only those peaks that were present in 80% of all biological samples in the entire dataset (this filtering step was reduced to 50% for the Zn and Ag lipidomics data). Peaks in the blank samples were removed from the sample spectra. The resulting matrices of peak intensities (termed “DIMS dataset”) were probabilistic-quotient normalised (PQN) and intensity-drift corrected using a Quality Control-Robust Spline Correction (QC-RSC) algorithm. Finally, the missing values were imputed using the k-nearest neighbours (KNN) algorithm. For multivariate analysis, generalized log (Glog) transformation of the DIMS dataset was also performed.

S1.4 RNA seq gene expression profiling

Total RNA was extracted from A549 cells using a micro RNeasy Kit (Qiagen, Crawley, UK) according to the manufacturer’s protocol. RNA was quantified with a NanoDrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA), and the integrity of RNA was evaluated with a Agilent 2200 Tapestation (Agilent Technologies, Santa Clara, CA). Only RNA with integrity numbers (RINs) greater than 7.0 were used for subsequent RNA-seq experiments. All RNA libraries were produced using the Biomek FxP (Beckman Coulter A31842) with Ultra Directional RNA Library Prep Kit (New England Biolab E7420L) and NEBnext Multiplex Oligos for Illumina Dual Index Primers (New England Biolabs E7600S), using provided protocols and 500ng of total RNA. Constructed libraries were assessed for quality using the Tapestation 2200 (Agilent G2964AA) with High Sensitivity D1000 DNA screentape (5067-5584), and quantified using Kapa Library Quantification Kit (Kapa Biosystems KK4824) on an AriaMx Realtime PCR System (Agilent

G8830A). Multiplex library clustering and sequencing was performed upon the HiSeq2500 with HiSeq Rapid Cluster Kit v2 (Illumina GD-402-4002) at 12pM library concentration with 10% PhiX Control v3 spiked in (Illumina FC-110-3001). The sequencing run was carried out using HiSeq Rapid SBS Kit v2 (Illumina FC-402-4021).

S1.5 RNA seq Data Processing

The BCL files were converted to FASTQ format using Illumina bcl2fastq conversion software (v1.8.4). Sequences were then trimmed using Trimmomatic (v0.36). FastQC (v0.11.2) was utilised to assess the data quality, and the QC results were summarised with MultiQC (v0.8). Five low quality samples were identified and removed accordingly. The remaining FASTQ files were aligned to the GENCODE human transcript sequences (release 25, GRCh38.p7) using Bowtie2 (v2.3.0). The resulting SAM data were converted into BED format using SAMtools (v1.3.1) and bamToBed (v2.19.1). Finally, the RNA read counts were extracted from the BED files with a Python script. To provide gene-level analysis, the RNA reads were collapsed to the counts of their coding genes. The gene annotation information was retrieved from the Ensembl database (release 87).

S1.6 Omics Data Analysis

Lipidomics and polar metabolomics peak intensity data were visualised in Genespring (v7.3.1; Agilent) and putative annotations were added using MI-Pack (Weber et al. 2011). ANOVAs and t-tests were performed in Genespring using multiple testing corrections (Benjamini and Hochberg 1995) for false discovery rate corrected p-values (FDR) of <0.05. Transcriptional data was mapped by gene. DESeq2 (Love et al. 2014) and used for differential expression analysis with a q<0.05 cut-off. Genespring was used to generate principal components analysis (PCA) scores plots from all omics datasets. Combined gene and metabolite pathway over-representation analyses were performed with IMPaLA (Kamburov et al. 2011), using gene identifiers and all Human Metabolite Database (HMDB) identifiers (Wishart et al. 2013) for each peak identified as significantly altered as input lists. The background sets were all detected transcripts and all identifiers annotated to detected lipid and polar metabolite peaks, respectively. Comparative pathway analyses were performed with Ingenuity Pathway Analysis (IPA; Qiagen) on combined sets of genes, lipids and polar metabolite identifiers using the mean fold change and FDR for each exposure group versus time-matched controls; analyses used only those identifiers with FDR<0.05. Raw data and experimental details are archived at ArrayExpress for transcriptomics (accession number E-MTAB-5734).

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