Project: Oxidative phosphorylation selectively orchestrates tissue macrophage homeostasis

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

Mouse colonies were bred at the CNIC under specific pathogen-free conditions and on C57BL/6 background. *Tfam*$^{f/f}$ (Larsson et al., 1998), *Ndufs4*$^{f/f}$ (Kruse et al., 2008) and *Sdhb*$^{f/f}$ (Cardaci et al., 2015) mice were kindly provided by Nils-Göran Larsson (Max Planck Institute for Biology of Ageing, Cologne, Germany), Richard D. Palmiter (University of Washington, Seattle, USA), and Eyal Gottlieb (Beatson Institute, Glasgow, UK), respectively. *Ndufs4*$^{f/-}$ mice were created by crossing floxed males with *Zp3*^{Cre} females (Lewandoski et al., 1997). All floxed mouse lines, as well as *Uqcrq*$^{f/f}$ (Weinberg et al., 2019) and *Cox10*$^{f/f}$ (Diaz et al., 2005) mice, were crossed with *CD11c*^{Cre} (Caton et al., 2007) or *LysM*^{Cre} mice (Clausen et al., 1999). Mice were group-housed, have not been used in previous procedures and were fed standard chow except for experiments using high fat diet (as indicated and see below). Littermates of the same sex were randomly assigned to experimental groups. Male and female mice were used for all experiments, expect experiments using high fat diet (as indicated and see below). Mice with the following ages were used for all experiments (as indicated): 2 days, 11 days, 3 weeks or 6–10-weeks (adult). The local ethics committee approved all animal studies. All animal procedures conformed to EU Directive 86/609/EEC and Recommendation 2007/526/EC regarding the protection of animals used for experimental and other scientific purposes, enforced in Spanish law under Real Decreto 1201/2005.

Tissue dissociation for cell isolation

Bronchoalveolar lavage (BAL) was performed by inserting a venal catheter (BD) into the trachea and 3-10 washes with 0.3-1 ml PBS and EDTA buffer to harvest BAL cells.

GC-MS untargeted metabolomics

Samples were prepared for the gas chromatography and mass spectrometry (GC-MS) untargeted metabolomics analysis by optimizing methods previously described (Mastrangelo et al., 2016). Briefly, 1mL of cold MeOH:H2O (9:1, v:v) was added to each sample containing 10^6 $CD45^+$ $F4/80^+$ $CD11c^+$ FACS-sorted AMs from the BAL of adult *Tfam*$^{f/f}$ and *CD11c*Δ*Tfam* mice. Samples were subjected to two freeze–thaw cycles for metabolism quenching and complete metabolite extraction, specifically by placing the samples at -80ºC for 15 min and thawing them on ice for 10 min with brief vortex-mixing. The samples were then centrifuged at 20,000 xg at 4°C for 10 min and the supernatant collected. The supernatant was evaporated to dryness (SpeedVac Concentrator, Thermo Fisher Scientific, Waltham, MA, USA) and derivatized with 10 µl O-methoxyamine hydrochloride (15mg/mL) in pyridine and 10 µl N,O-bis(trimethylsilyl)trifluoroacetamide in 1% trimethylchlorosilane. Finally, 100 µl of heptane containing 10 ppm of 4-nitrobenzoic acid (IS) was used as internal standard to monitor sample injection. For data acquisition, 7250 GC/Q-TOF using the electron ionization (EI) source was used; separation was carried out using a J&W guard column (10 m x 0.25 mm, 0.25 µm), integrated with a DB5-MS column (30 m x 0.25 mm, 0.25 µm film, Agilent Technologies). Metabolite deconvolution and identification were carried out using Agilent MassHunter Unknowns Analysis version B.07.00, then, data was aligned in Agilent Mass Profiler Professional version B.12.1 and exported to Agilent MassHunter Quantitative Analysis version B.07.00. Metabolites were

identified by comparing their retention time, retention index and mass fragmentation patterns with those available in an in-house library including both the NIST mass spectral database (version 2017) and Fiehn RTL library (version 2008). The different derivatives that were generated from the silylated compounds were unified by summing the abundance of all derivatives from the same metabolite. Finally, the median relative area of the two analytical replicates of the same sample was computed and used for subsequent statistical analysis. The result was a matrix with the compounds in the samples sorted by their characteristic retention time and target ion, and the relative abundance of each compound for each sample.

Quality Control (QC) samples (n=4) were prepared by pooling equal volumes of cell extracts from each sample by following protocols mentioned above. QC samples were injected at the beginning, at the end and every six samples in order to assess the reproducibility of both sample preparation and data acquisition. Raw data from all samples were processed as described above. Data quality was assured by using the QC samples as reference by filtering the data matrix. Specifically, metabolites present in 50% of the QC samples with a coefficient of variation below 50% were retained and the resulting data matrix underwent principal component analysis (PCA) on Metaboanalyst website (http://www.metaboanalyst.ca/) to assess the analytical reproducibility.

Cube root transformed metabolites' levels from knockout and wild type mice were compared using Student's t test and the p-values adjusted using the Benjamini–Hochberg method correcting for false discovery rate (FDR, q=0.05). Differences were considered statistically significant when $p \leq 0.05$. Individual samples (n=3 per each genotype) were generated by merging FACs-sorted AMs to 10^6 cells/sample from >10 independent experiments of more than 13-30 animals/genotype.