Quantitation of endogenous metabolites in mouse tumors using mass-spectrometry imaging

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Supplementary Information

Quantitation of endogenous efficacy markers in mouse tumors using mass spectrometry imaging

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Tissue Embedding

In order to minimize any potential degradation of the endogenous analytes, all tumors from each model were embedded together in a plastic mould (VWR International Ltd, Lutterworth, Leicestershire, UK) using 10% w/v gelatin solution. Gelatin blocks were stored at -80ºC prior to cryosectioning.

Sectioning

Gelatin embedded tissues were cryosectioned on a CM3050S cryomicrotome (Leica Biosystems, Nussloch, Germany) at a thickness of 10 µm and thaw mounted onto indium tin oxide (ITO) coated MALDI target slides (Bruker Daltonics, Bremen, Germany). Once thaw mounted slides were immediately dessicated using a stream of dry N_2 prior to vacuum packing and storage at −80°C until analysis.

Kidney sections used in the stability experiment were sectioned onto Superfrost glass slides (Fisher Scientific, Loughborough, Leicestershire, UK) for subsequent DESI analysis.

Tissue sections were taken at approximately equal depth from all samples.

Matrix application

Vacuum packed, thaw mounted tissue sections were allowed to reach room temperature after removal from -80°C storage, prior to breaking the vacuum seal. Optical images were taken using a standard flat bed scanner (Seiko Epson, Negano, Japan) prior to MALDI matrix application. Matrix coating was applied using a TMsprayer (HTX technologies, Chapel Hill, NC, USA) set at 75°C and perfoming 8 passes, with a back-up flow of 50% methanol/water *v/v* at a flow rate of 0.08 mL/min and nebulized with nitrogen at 8 psi (lactate) or 6 psi (glutamate). Either 1,5-Diaminonaphthalene (10 mg/mL, 50/50 *v/v* acetonitrile/water,) containing 100 µL of Sodium L-lactate-^{13}C1 solution (Sigma-Aldrich, Loughborough) was

used for lactate quantitation and 9-aminoacridine (5 mg/mL, 70/30 *v/v* acetonitrile/water) was applied using the same conditions as the internal standard (D5-glutamate) for glutamate quantitation.

DESI Mass Spectrometry Imaging

DESI MSI analysis was performed using a Q-Exactive mass spectrometer (Thermo Fisher Scientific Inc., Bremen, Germany) operated in negative ion mode. The mass spectrometer was equipped with an automated Prosolia 2D DESI source (Indianapolis, IN, USA). Mass spectra were collected in the mass range of *m/z* 80-600 at a mass resolution of 70,000 (at *m/z* 200). Methanol/water (95:5 v/v) was used as the electrospray solvent held at 4.5kV spray voltage and delivered at a flow-rate of 1.5 μL/min by a Dionex Ultimate 3000 nanoLC pump (Thermo Fisher Scientific). Nitrogen was used as nebulizing gas at a pressure of 7 bars. The height distance between the DESI sprayer and the sample surface was set to 1.5 mm with the distance between the sprayer and the inlet capillary set to 7 mm. The distance between the sample surface and the inlet capillary of the mass spectrometer was < 1 mm. The angle between the sprayer tip and the sample surface was set at 75°. Spatial resolution for the imaging experiment was set to 100 μm with 371.67 μm/s scan speed at an injection time of 250 ms per spectrum. Individual horizontal line scans were combined into imzML format using the imzML converter V.1.1.4.5 (www.maldi-msi.org). Data visualization was performed using MSiReader v0.09[26]. Intensity data was extracted for each pixel within a region of interest and subsequently averaged using Microsoft Excel. All images were created using 0.01 Da bin size and linear interpolation (order 1).

Homogenization and Quantitation of Lactate Levels by LC-MS

Whole tissue lysates was obtained after homogenization and extraction with 1 mL ACN/MeOH/H2O v/v/v per 50 mg of tissue on Precellys 24 temperature controlled device using CKMix50R-2 ml tubes. Tissue homogenization was completed after 3*20second cycles at 5000rpm with 30 seconds pause in between. After centrifugation at 0ºC for 5 mins at 10621 g (Eppendorf 5417R) a maximum of 700 µL clear supernantant was placed in an eppendorf tube and the extraction procedure was repeat as described above. The second fraction of clear supernantant was combined in the same eppendorf tube with the first extract before storing at -20ºC.

Prior to LC-MS analysis 5 µL of tissue extract was diluted with 45 µL of 55 µM $^{13}C_3$ Lactic acid (Dilution A). 5µL of diluent A was mixed in a polypropylene HPLC vial with 45 µL H_2O, followed by quick vortex and 10min centrifugation at 3273 g (4ºC).

Lactic acid was chromatographically resolved from matrix interferences under isocratic elution condition with 20% eluent B (Eluent A: 10 mM tributylamine, 15 mM acetic acid in H_2O, Eluent B: Isopropanol/MeOH 20/80 *v/v*) and quantitation results were obtained over a linear range of 0.5-160 µM concentration. Batch validation completed on quality control samples (QC) prepared by mixing equal aliquots of all tissue lysate and spiked with $^{13}C_3$ Lactic acid at concentration of 0.5, 1, 5, 40 and 120 µM. Validation QCs at each concentration were injected at the beginning, middle and end of the analytical batch to obtain accuracy (<7% bias) and precission (<7% CV) values.