Supplementary Material

Adrenal Hormone Interactions and Metabolism: A Single Sample Multi-omics Approach

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1. Development of a single sample preparation procedure for multi-omics analysis

Based on an existing methodology for measurements of enzyme activity in cells and tissues requiring the isolation of native proteins, a special cell disruption buffer was tested for further suitability for extractions of both polar and non-polar compound classes within the same sample [1]. Therefore, several sample materials including cell culture, mouse adrenals and tumor tissue specimens were lysed and homogenized in cell disruption puffer (Figure S1). The resulting lysates were aliquoted and diluted with the appropriate additives required for the respective compound classes (Table S1).

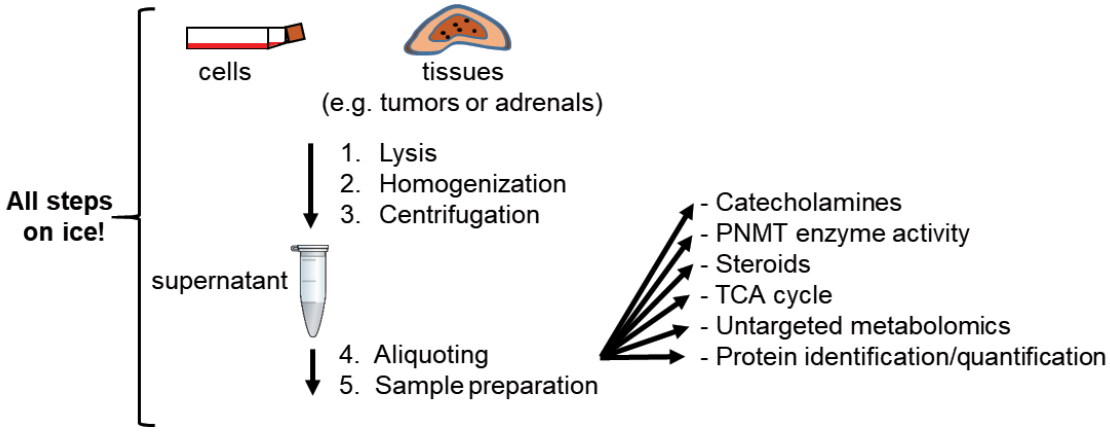

Figure S1: Sample preparation procedure allowing multi-omics analyses in different sample matrices.

Additives were similar to the digestion agents used in conventional sample preparation. The volume of lysate used for the dilution and the following analyses was defined according to the respective detection limits of the following methods and the expected amount of metabolites in the sample. The developed protocol allows for the first time the analysis of different analytes, polar and non-polar metabolites as well as proteins, from a single sample.

Table S1: Pipetting scheme for processing of lysates for analyses.

analytical method	additive	volume of additive [µl]	volume of lysate [µl]
catecholamines	perchloric acid	90	10
steroids	methanol	85	15
PNMT enzyme activity	-	-	40
TCA cycle metabolites	methanol	85	15
untargeted metabolomics	methanol	85	15
protein quantification	deionized water	45	5

2. Validation of the novel procedure in pheochromocytoma cells

2.1. Cell culture work

For samples derived from cell culture experiments, MPC mouse pheochromocytoma cells (MPC 4/30/PRR) [2] and PC12 rat pheochromocytoma cells [3] were cultivated as described elsewhere [4]. Cells (4×10^6) were seeded in T75 cell culture flask (Greiner). After 48 h incubation time under normoxic conditions (37°C, 5% CO_2, and 95% humidity), adhered cells were trypsinized (trypsin / EDTA; 0.05% / 0.02%), diluted with complete medium and counted using C-CHIPs (Neubauer improved). Cell suspensions containing 5×10^5 cells were transferred to pre-cooled sample tubes, centrifuged for 5 min at 4°C (16,000 x g) and washed three times with cold PBS. The obtained cell pellets were stored at -80°C until further processing.

2.2. Results

Cellular contents of catecholamines and TCA-cycle metabolites in MPC and PC12 cells were analyzed using both conventional and novel preparation procedures. In both cell lines, catecholamines, dopamine and norepinephrine were detected in similar concentrations irrespective of the preparation procedure (Figure S2). The catecholamine precursor DOPA was only detectable in MPC cells, whereby the preparation procedure had no influence on the analyzed concentration. For TCA-cycle metabolites, citrate, fumarate and malate, no changes were observed using either the novel or the conventional procedure in both cell lines (Figure S2). α-ketogluterate was only detectable in MPC cells and the comparison of both methods demonstrated that the application of the single sample procedure led to an increased concentration of α-ketogluterate (91.6% change, $p=0.003$). For the metabolites, cis-aconitate, isocitrate, succinate and lactate the use of the novel procedure also resulted in a 84.1 and 93.9%-change ($p=0.015$, $p=0.008$), 97.3 and 98.6%-change ($p<0.001$, $p<0.001$), 37.8 and 46.3%-change ($p=0.004$, $p<0.001$), 68.3 and 44.6%-change ($p<0.001$, $p<0.001$) in the metabolite concentration of PC12 and MPC cells compared to the conventional procedure (Figure S2). Thus, the newly established sample preparation led to similar or even increased concentrations of the analyzed TCA-cycle metabolites in the cells compared to the conventional method. The amino acids aspartate, asparagine, glutamate and glutamine showed no differences between the two preparation procedures (Figure S2). Observed changes in the metabolite concentration followed the same direction in both cell lines, which confirms that observed differences are not due to cell line- or matrix-dependent effects.

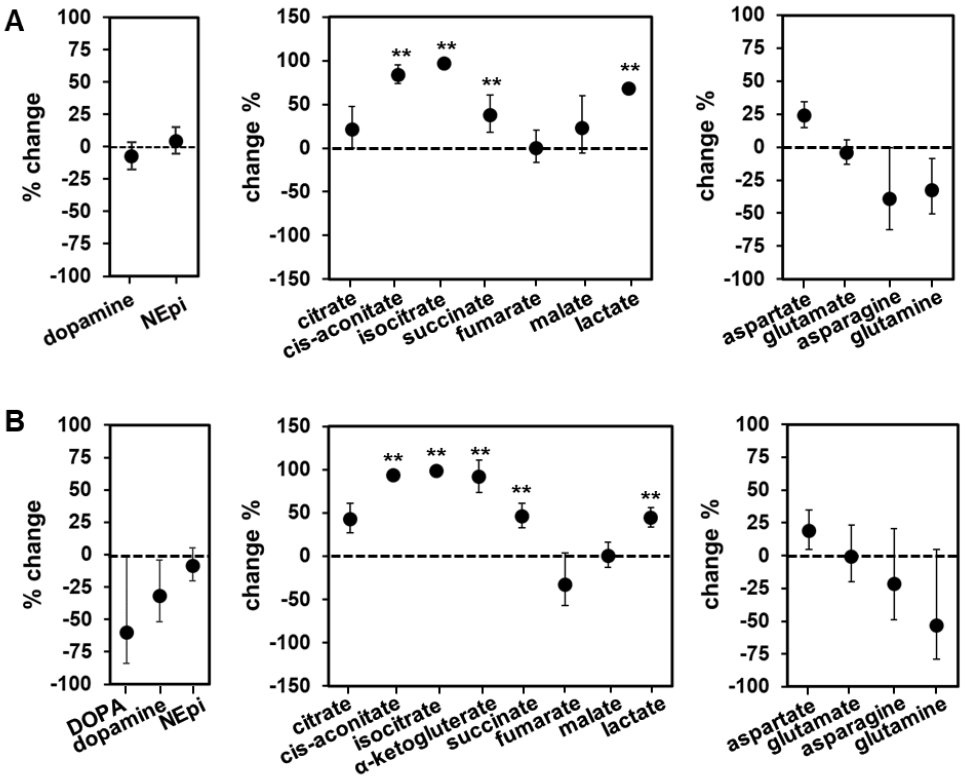

Figure S2: Changes in the metabolite concentration after applying the novel and conventional preparation protocols on cell pellets of two different pheochromocytoma cell lines. (**A**) PC12 and (**B**) MPC cell pellets were either prepared using the novel or the conventional procedure. The metabolites are presented as mean of percentage change with 95% confidence interval (CI). The mean as well as the CI were calculated after logarithmic transformation of the fold change and afterwards re-transformed (antilog) as percentage. Positive changes indicate higher metabolite concentrations obtained with the novel procedure. DOPA and α-ketogluterate were not detectable in PC12 cells. Wilcoxon signed rank test, $^{**}p<0.01$.

3. TCA-cycle metabolites in pheochromocytoma and paraganglioma tissues

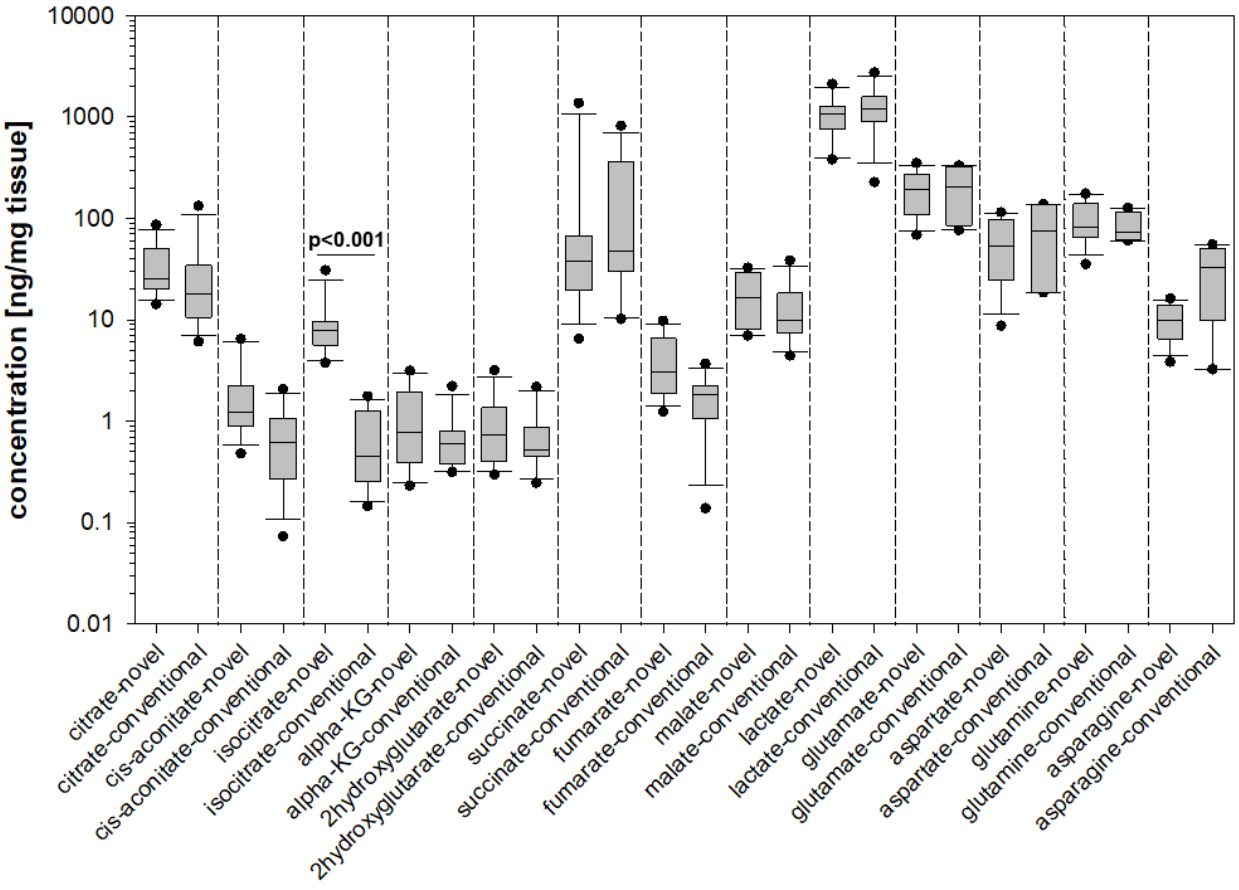

Figure S3: Results of TCA-cycle metabolites and amino acids derived from tumor tissue samples using the novel and the conventional preparation procedure. Both procedures resulted in comparable metabolite concentrations, except for isocitrate. Significance of differences after post-hoc Bonferroni correction.

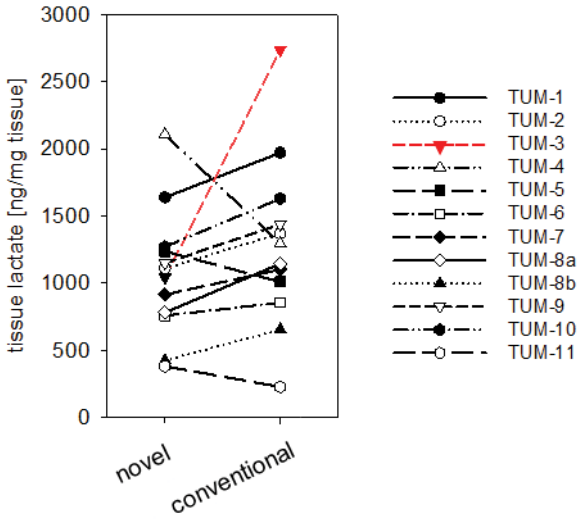

Figure S4: Lactate concentrations observed in tumor tissue derived samples using the novel and the conventional preparation procedure. TUM-3 highlighted in red showed an extensive differences between both procedures.

Reference

1. Qin N, Peitzsch M, Menschikowski M et al. Double stable isotope ultra performance liquid chromatographic-tandem mass spectrometric quantification of tissue content and activity of phenylethanolamine N-methyltransferase, the crucial enzyme responsible for synthesis of epinephrine. Analytical and bioanalytical chemistry. 2013;405(5):1713-9.
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