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Oncogene induced shifts in metabolic flux of bi-clonal tumours as revealed by stable isotope labeling and mass spectrometry imaging (MSI) analysis

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Introduction: The role of MSI in studying tumour metabolism

Tumour metabolism is distinct from that of healthy tissues, typically characterized by an increase in biosynthetic pathways, which is sided by increased nutrient flux but also by the occurrence of aerobic glycolysis. Importantly, the precise metabolic shifts seen in oncogenic tissues are governed by the underlying mutational landscape and the driving oncogenes.

All known tumours show some degree of heterogeneity, driven by clonal evolution, competition, but at times also synergy between clones. Traditionally, the metabolisms of such inherently multiclonal tumours was measured by bulk analysis, thus representing an average metabolism of all individual components.

The advent of MSI is thus a major advancement in cancer biology, as the metabolism of different clones can be measured *in-situ*.

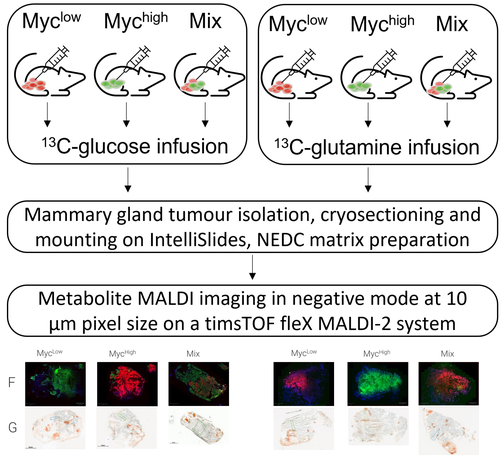


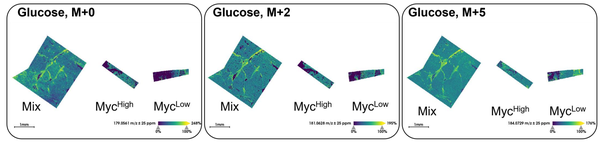
Fig. 1 Method setup for metabolic flux analysis in a bi-clonal model for mammary gland tumours by MALDI imaging. Fluorescence images of consecutive sections (F) and grayscale scans of sections for MALDI Imaging with indicated measurement areas (G).

Methods

We generated a traceable bi-clonal breast cancer model, harbouring Myc^{High} and Myc^{Low} clones, which are marked with green and red fluorescent proteins respectively (Fig. 1). Uniformly labelled ^{13}C-glucose or ^{13}C-glutamine were administered as tracers.

The tumours were cryosectioned and NEDC matrix was sprayed with an HTX-sprayer. MALDI imaging data was acquired on a timsTOF fleX MALDI-2 instrument with or without postionization at 10 µm pixel size in negative Q-TOF mode. The instrument was tuned for a mass range ranging from 50-650 m/z. Calibration was performed externally using red phosphorous. SCiLS Lab version 2021c was used for data analysis and visualization after RMS normalization.

[$^{13}C_6$]-glucose infusion



[$^{13}C_5$]- glutamine infusion

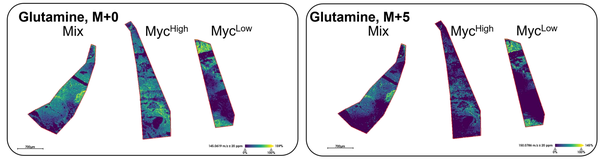


Fig. 2. After [$^{13}C_6$]-glucose infusion (upper panel), we observed glucose as M+0, M+2 and M+5 in all cell types, but with an interesting accumulation in tumour associated stromal cells. Glutamine was detected as unlabeled and completely labeled variant after [$^{13}C_5$]-glutamine infusion (lower panel).

Results

- Stable isotope labeled tracers (glucose and glutamine) were detected in the different cell types by MALDI imaging (Fig.2).
- Intermediates of the glycolysis and TCA-cycle were detected with different degrees of incorporated labeling (Fig. 3, 4, 5, 6).
- The different cells types accumulated different amounts of heavy isotope labeling in the TCA-cycle intermediates malate and succinate suggesting variation in the TCA-cycle activity (Fig. 5 and 6).
- MALDI-2 postionization increased the sensitivity for the detection of malate (Fig. 5) and succinate (Fig. 6).

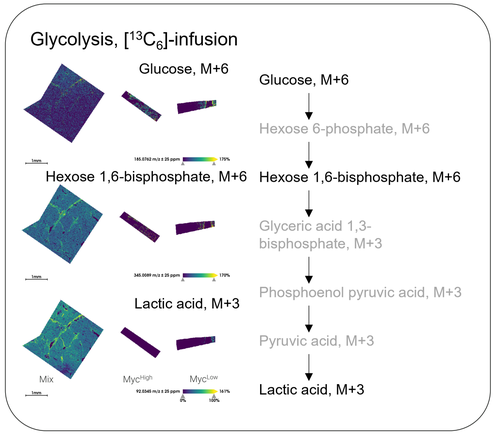


Fig. 3 Detected fully labeled compounds of the glycolysis and lactic acid fermentation after [$^{13}C_6$]-glucose infusion were tentatively observed mainly in stromal areas.

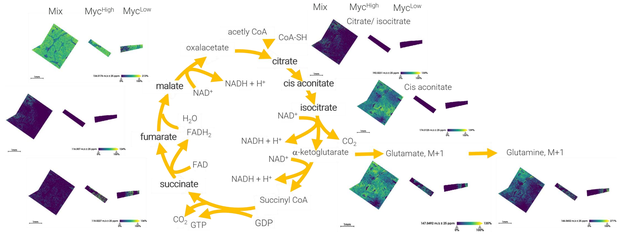


Fig. 4 TCA-cycle with MALDI images of intermediates detected as putative M+1.

The aim of this study was to test the applicability of MALDI mass spectrometry imaging to perform metabolic flux analyses in a bi-clonal tumour model.

With high spatial resolution (10 µm) and MALDI-2 postionization, we could detect various metabolites of the glycolysis and TCA-cycle. Interestingly, we found different degree of labeling in different cell types, suggesting different metabolic activities in tumour tissue compared to tumour associated stromal cells.

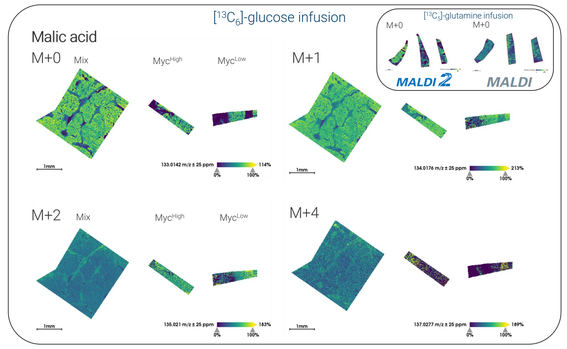


Fig. 5 . Malic acid after [$^{13}C_6$]-glucose or [$^{13}C_5$]-glutamine infusion (inlet). High levels of malate M+0 and M+1 suggesting increased TCA-cycle activity were found in tumour tissue, while higher label accumulation (M+2) was seen in stromal cells, arguing for a more divers carbon source in the tumour tissue.

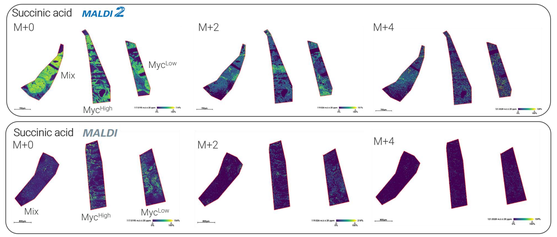


Fig. 6 . Succinic acid was detected as M+0, M+2 and M+4 after [$^{13}C_5$]-glutamine infusion with increased sensitivity when data was acquired with MALDI-2 postionization.

Conclusion

- Metabolic flux analysis can be accomplished by MALDI mass spectrometry imaging.
- MSI can detect differences in metabolic acitivities of cells in their spatial environment.
- MALDI-2 postionization can increase the sensitivity for the detection of TCA-cycle intermediates in MSI.

Technology