Deciphering Metabolic Heterogeneity in Lung Cancer Cell Lines Using High-Resolution MALDI Mass Spectrometry Imaging

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Introduction

Lung cancer is a highly heterogeneous disease shaped by genetic alterations and a complex tumor microenvironment (TME). Among immune cells in the TME, macrophages are the most abundant and play a central role in tumor progression through metabolic crosstalk with cancer cells, promoting immunosuppression and metabolic reprogramming. These interactions drive both inter- and intratumoral metabolic heterogeneity. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI Imaging) enables spatially resolved analysis of metabolites and lipids at near single-cell resolution, providing a powerful tool to study cell-cell metabolic interactions. By directly mapping metabolic alterations within defined co-culture systems, MALDI Imaging offers unique insights into how macrophage-tumor interactions shape cancer metabolism and immune phenotypes in lung cancer. These high-resolution approaches enable the identification of phenotype-specific metabolic signatures that are otherwise obscured in bulk analyses. In particular, resolving macrophage heterogeneity at the single-cell level allows a more precise characterization of functional states within the tumor microenvironment.

Methods

Single cell spatial metabolomics was performed using MALDI Imaging on mono- and co-cultures of human peripheral blood mononuclear cell (PBMC)–-derived or THP1 (human monocytic cell line)-derived macrophages with A549 lung cancer cells (Fig.1). On ITO-coated IntelliSlides®, PBMCs were differentiated into macrophages using 20 ng/mL MCSF or human serum over 10 days, while THP1 cells were differentiated using 10 ng/mL PMA for 24 h followed by a 24 h recovery. Macrophages were polarized toward pro- or anti-inflammatory phenotypes using LPS+IFN-γ or IL-4, respectively. After vacuum-drying, NEDC matrix was applied by sublimation using a Bruker **super**limator™. MALDI Imaging was acquired on a timsTOF fleX MALDI-2 at 5 µm spatial resolution and analyzed using SCiLS™ Lab and MetaboScape®. Immunofluorescence staining (IF) was additionally performed to improve cell segmentation.

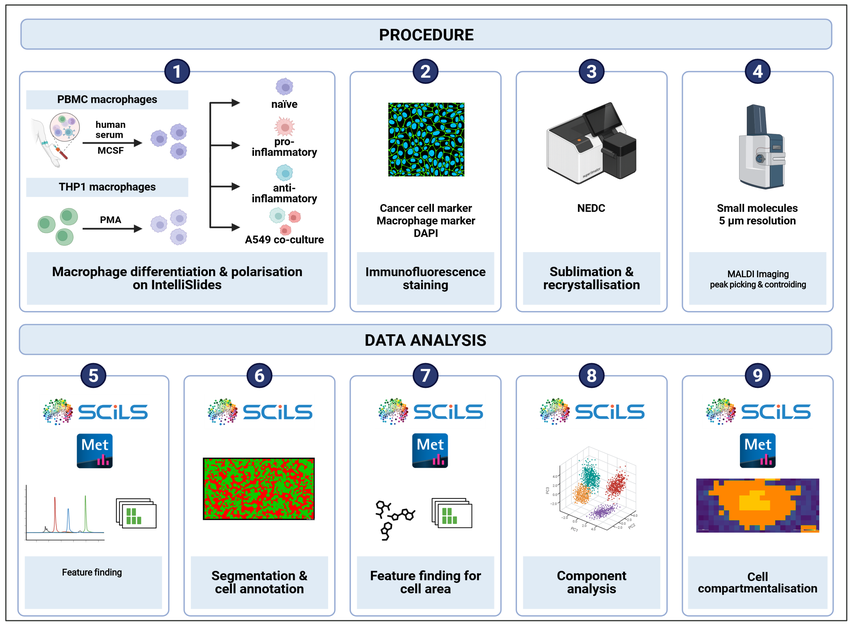

Fig. 1 Workflow for ThP1 and human PBMC-derived macrophage isolation, MALDI Imaging and data analysis

Results

Matrix application by sublimation using the Bruker **super**limator enabled artifact-free, high-resolution MALDI Imaging without analyte delocalization or cellular leakage. Optimized conditions produced clean spectra with high signal intensity for both lipids and metabolites, allowing robust discrimination of individual cell types within co-culture systems. Adenine was used as a nuclear marker to facilitate single-cell segmentation. Furthermore, we employed various lipid species including linoleic, oleic and stearic acid to enhance cellular segmentation to cover most likely cytoplasm area in addition to adenine (Figure 2A). With this method, nucleotides (ATP), amino acids (Oxoproline), lipids (arachidonic acid) and TCA cycle metabolites (succinate) were detected in single cell resolution in PBMC-derived macrophages (Figure 2B).

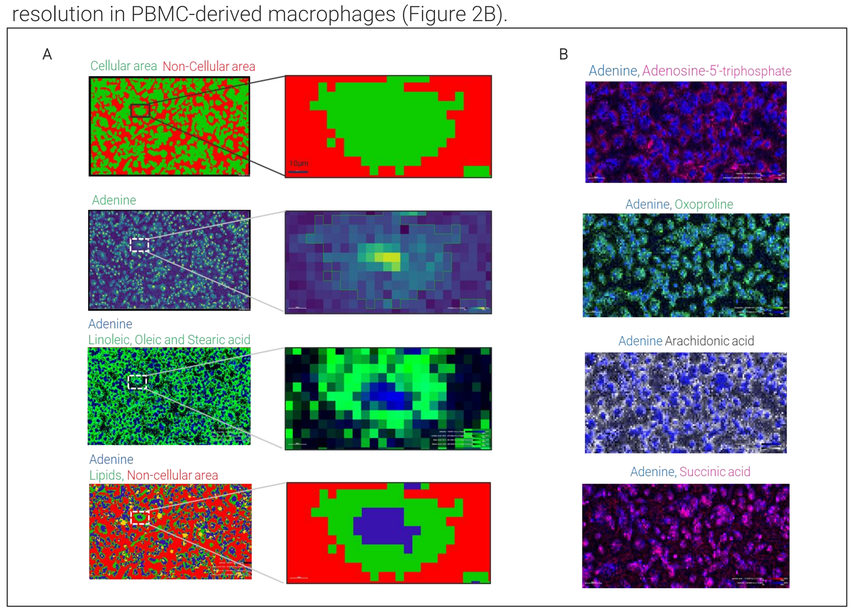

Fig. 2 Human-PBMC derived metabolic segmentation and metabolic profile. (A) cellular segmentation of human PBMC-derived macrophages based on metabolic profile guiding by adenine and lipids. (B) Metabolic profile of naïve PBMC-derived macrophages covering nucleotide, amino acid, lipid and TCA cycle metabolites

Among the most prominently regulated mitochondrial metabolites, pro-inflammatory macrophages exhibited significantly elevated levels of adenosine triphosphate (ATP), indicating altered nucleotide metabolism associated with inflammatory signaling. In contrast, anti-inflammatory macrophages displayed altered glutamine metabolism, characterized by significantly elevated levels of oxoproline and glutamate. Notably, naïve macrophages co-cultured with A549 lung cancer cells showed reduced ATP levels compared with pro-inflammatory macrophages, further suggesting that cancer cell interactions modulate macrophage energy metabolism and inflammatory status (Fig 3). Within the tricarboxylic acid (TCA) cycle, succinic acid was significantly elevated in pro-inflammatory macrophages compared with naïve and anti-inflammatory phenotypes. In addition, malic acid levels were higher in naïve macrophages compared with anti-inflammatory macrophages. Co-culture with A549 lung cancer cells reduced both succinic acid and malic acid levels in naïve macrophages, further supporting the notion that TCA cycle metabolism exhibits distinct metabolic signatures across macrophage phenotypes and can be reprogrammed by cancer cell interactions.

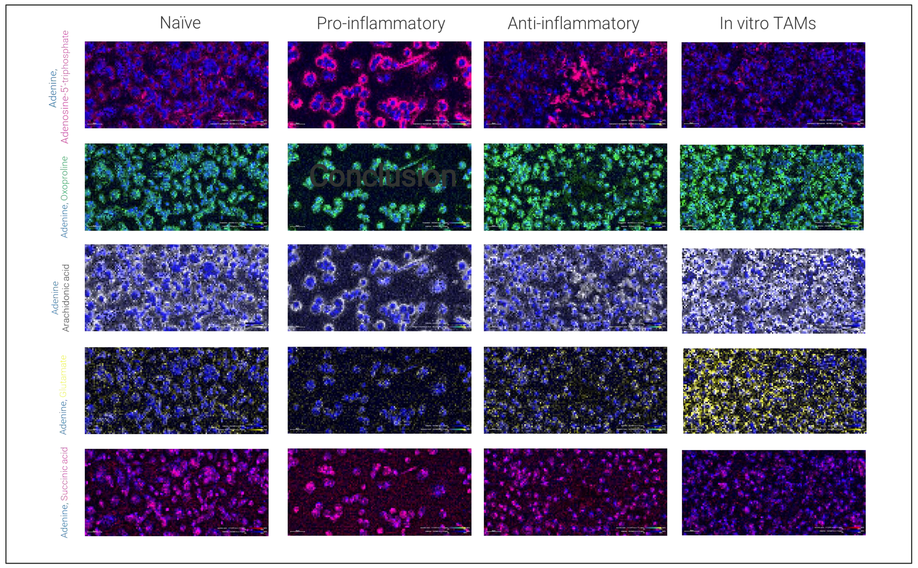

Fig. 3 Metabolic profile of PBMC-derived macrophages in naïve, anti-inflammatory, pro-inflammatory and after co-culture with A549 lung cancer cells (in vitro TAMs)

Summary

Sublimation-based matrix application with the **super**limator enabled artifact-free, high-resolution MALDI Imaging, allowing reliable single-cell metabolic profiling in macrophage–cancer co-cultures. Distinct metabolic signatures were observed across macrophage phenotypes: pro-inflammatory cells showed increased ATP and succinate, while anti-inflammatory cells exhibited enhanced glutamine metabolism. Co-culture with A549 lung cancer cells altered macrophage metabolism, reducing TCA intermediates and shifting naïve macrophages toward an anti-inflammatory-like state. Overall, the data highlight dynamic metabolic crosstalk between macrophages and cancer cells, detectable at single-cell resolution.

Conclusion

- 5 µm MALDI Imaging enables near single-cell metabolic resolution
- Macrophage states differ metabolically and shifted in the presence of cancer cells
- Cancer cells induce an anti-inflammatory metabolic profile in naïve macrophages
- Sublimation matrix → artifact-free, high-quality data

Imaging MS: Pharmaceuticals, Metabolites, Lipids, and Glycans

COI Disclosure: T.B., C.H., J.O., A.K., M.E. and J.H. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study