Mass Spectrometry Imaging of Colorectal Cancer

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Introduction

Colorectal cancer (CRC) is among the leading causes of cancer-related deaths worldwide, with incidence expected to rise in the coming decades[1]. Increasing attention has been given to intra-tumor heterogeneity, as spatial and genetic variability within tumors may influence metastatic potential[2].
Metabolic reprogramming supports tumor survival in hypoxic conditions. While anaerobic glycolysis is well characterized, lipid metabolism in CRC remains less investigated, though several lipid species, including phosphatidylcholine PC16:0/16:1, have been identified as potential biomarkers[3,4]. Techniques capable of mapping lipid spatial distribution and integrating these findings with clinical parameters are crucial for understanding CRC biology and identifying new therapeutic targets.
Mass spectrometry imaging (MSI) enables label-free visualization of lipids, proteins, and metabolites directly in tissue sections, combining molecular and spatial information. By correlating MSI data with histology, tumor heterogeneity can be characterized more precisely[5].

Objectives

- To develop and optimize MSI methods for the analysis of CRC, with particular emphasis on the integration of molecular data with histopathological features of tumor tissue.
- To correlate MSI-derived molecular profiles with histological analyses, including H&E staining and IHC, in order to establish precise associations between molecular signatures and structural as well as functional characteristics of the tumor microenvironment.
- To identify specific regions of interest (ROIs) within tumor tissue and to conduct differential analyses between distinct tissue structures, providing an in-depth understanding of tumor heterogeneity, cellular composition, and potential biomarker identification.

Methods

- MSI experiments were performed using Waters SynaptXS mass spectrometer equipped with a nano-DESI ion source in positive and negative ion mode.
- Data processing was carried out using HDI software.
- Mass Vision, hosted on the 3D Slicer image computation platform, was used for global PCA analysis, data visualization, and correlation of MSI data with corresponding histopathology images.
- Differences between distinct structures within tumor tissue were assessed through region of interest (ROI) analysis.
- Database searches and statistical analyses were performed using MetaboAnalyst 6.0.
- Hematoxylin and eosin (H&E) staining of consecutive cuts
- Ki67-IHC staining after MSI.
- This research was approved by the institutional Ethics Committees of the Clinical Hospital Dubrava (Zagreb) and Clinical Hospital Center of Rijeka. All donors provided their written informed consent to participate in this study.

Principle of MSI

Molecule ionization is performed *in situ* at defined grid points (pixel) on the sample surface. Mass spectra are collected at each pixel and subsequently used to generate heat map images showing the distribution of specific molecules across the sample (Figure 1).

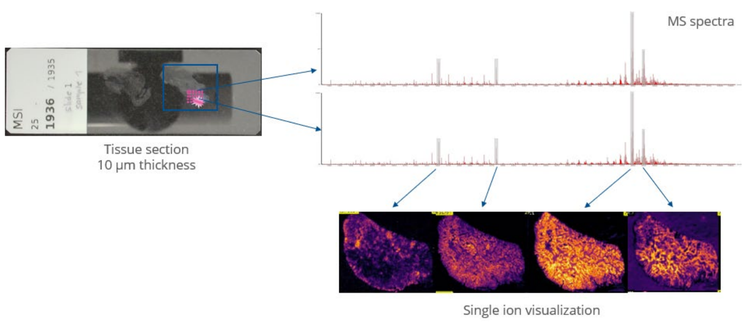

Figure 1. The principle of MSI.

Conclusions

- MSI methodologies for CRC analysis were successfully developed and optimized, allowing integration of molecular data with histopathological features of tumor tissue.
- Correlations between MSI-derived molecular profiles and histological analyses, including H&E and IHC, enabled precise mapping of molecular signatures onto the structural and functional characteristics of the tumor microenvironment.
- Identification of key regions of interest (ROIs) and differential analyses between distinct tissue structures provided deeper insights into tumor heterogeneity, cellular composition, and potential biomarker candidates, highlighting the translational value of MSI in colorectal cancer research.
- Future experiments will focus on validating these findings across larger patient cohorts, enabling robust assessment of biomarker reproducibility and their potential clinical relevance.

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3) Harewood R, Rothwell JA, Bešević J et al. Association between pre-diagnostic circulating lipid metabolites and colorectal cancer risk: a nested case–control study in the European Prospective Investigation into Cancer and Nutrition (EPIC). BioMedicine 2024;101: 105024, https://doi.org/10.1016/j.ebiom.2024.105024

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Hematoxylin and eosin (H&E) staining

Hematoxylin and eosin (H&E) staining remains the gold standard in histopathology for visualizing cellular and tissue architecture in CRC. It allows pathologists to accurately annotate the tumor microenvironment, including the distribution of different cell types and structural features (Figure 2). However, H&E staining provides limited insight into intracellular metabolic activity and lipid composition, which are increasingly recognized as important factors in tumor biology and therapeutic response.

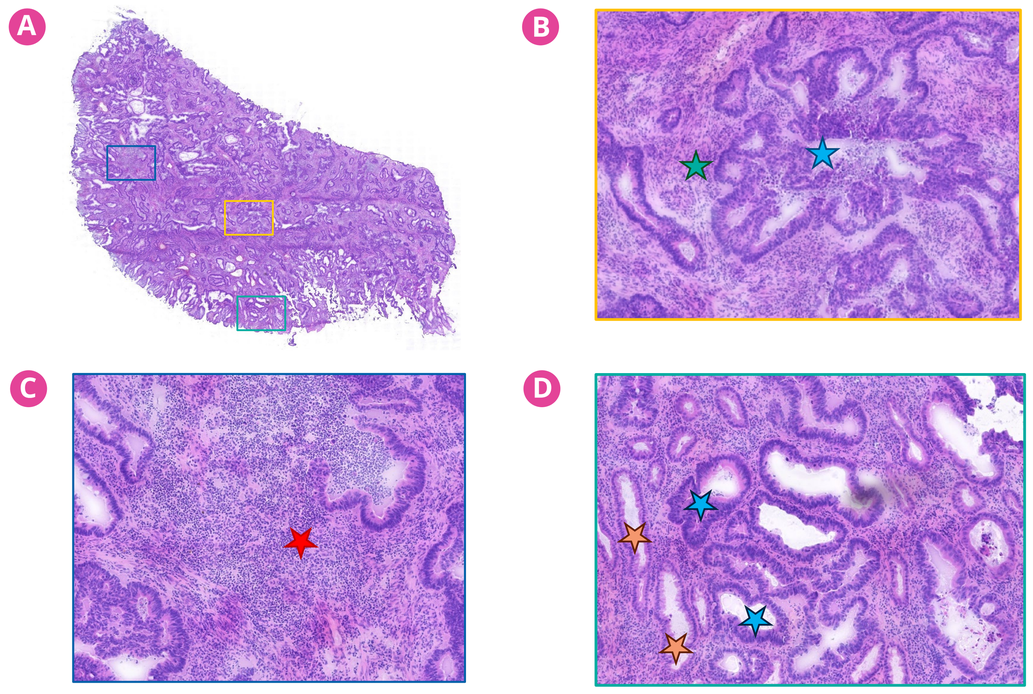

Figure 2. CRC tumor tissue after H&E staining (a); colored squares indicate the areas shown in the zoomed views in (b–d). Zoomed-in views with annotated structures: (b) Tumor stroma (green star) and neoplastic glands (blue star); (c) 'Dirty necrosis' with acute inflammatory cell infiltrate (red star); (d) Non-dysplastic glands (orange stars) and neoplastic glands (blue stars).

Mass spectrometry imaging

PCA analysis of MSI data allowed molecular level visualization of structures corresponding to those observed in H&E images, thereby facilitating a direct comparison between molecular and morphological tissue characteristics (Figure 3).

Differential expression analysis revealed numerous endogenous molecules with altered abundance between annotated tissue structures (Figure 4), including *m/z* 770.5026 ([M+K]·) corresponding to phosphatidylcholine PC16:0/16:1 that was more abundant in neoplastic glands in comparison to tumor stroma (Figure 5).

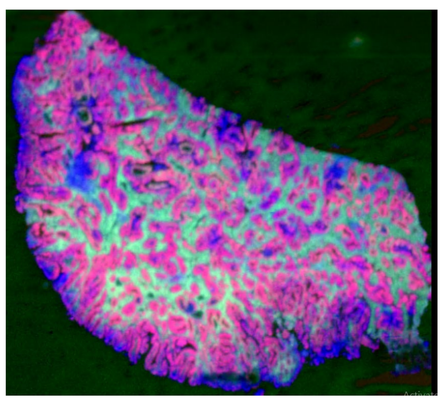

Figure 3. PCA analysis of MSI data. Colors indicate the distribution of different PCA components.

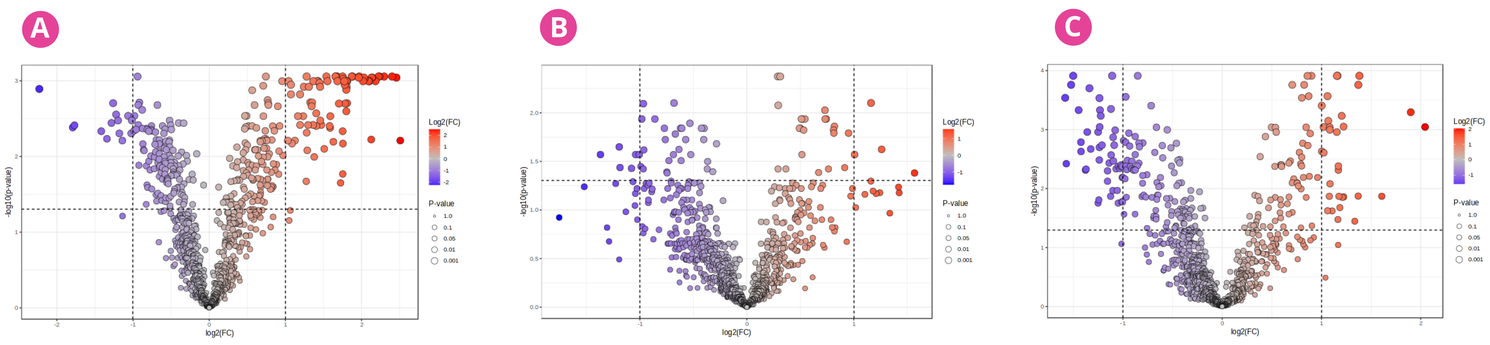

Figure 4. Differential expression analysis results. (a) Tumor stroma vs dirty necrosis, (b) neoplastic vs non-dysplastic glands and (c) tumor stroma vs neoplastic glands.

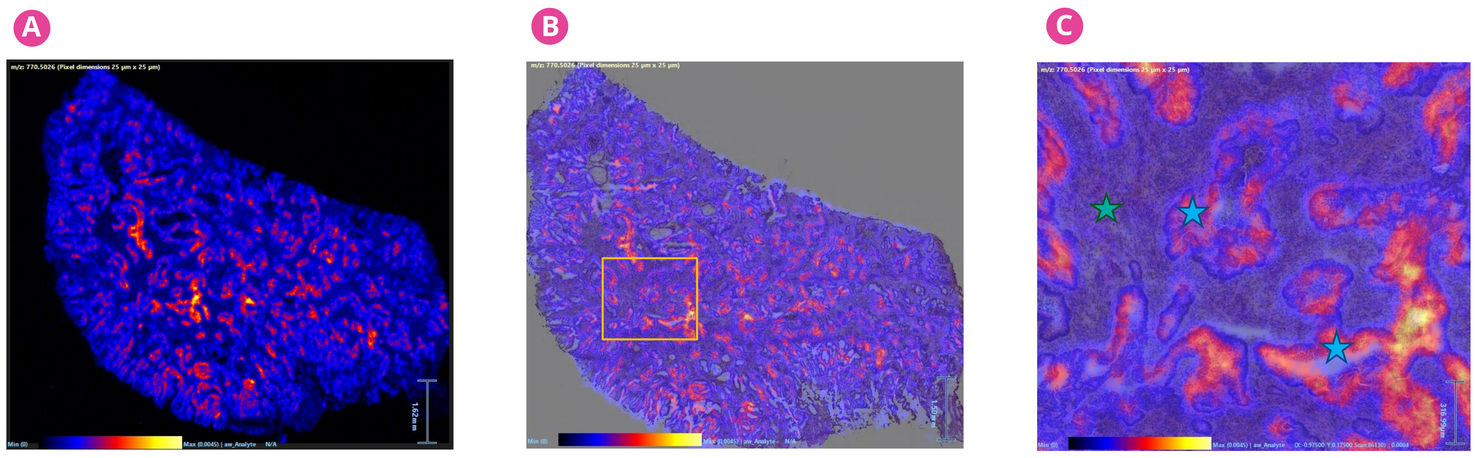

Figure 5. Spatial distribution of phosphatidylcholine PC16:0/16:1 in human CRC. (a) Representative ion image of *m/z* 770.5026 ([M+K]⁺) detected in the CRC tissue section. (b) Overlay of the ion image with the corresponding histological image (Figure 1a); the white square indicates the area shown in the zoomed view in (c). Tumor stroma is indicated by green star, while neoplastic glands are indicated by blue stars.

Mass spectrometry imaging and immunohistochemistry (IHC)

MSI is a nondestructive technique, preserving tumor tissue architecture and thereby allowing subsequent IHC staining of the same section after the MSI experiment. Figure 6 shows MSI data of healthy intestinal epithelium and CRC tissue alongside IHC results obtained after MSI acquisition.

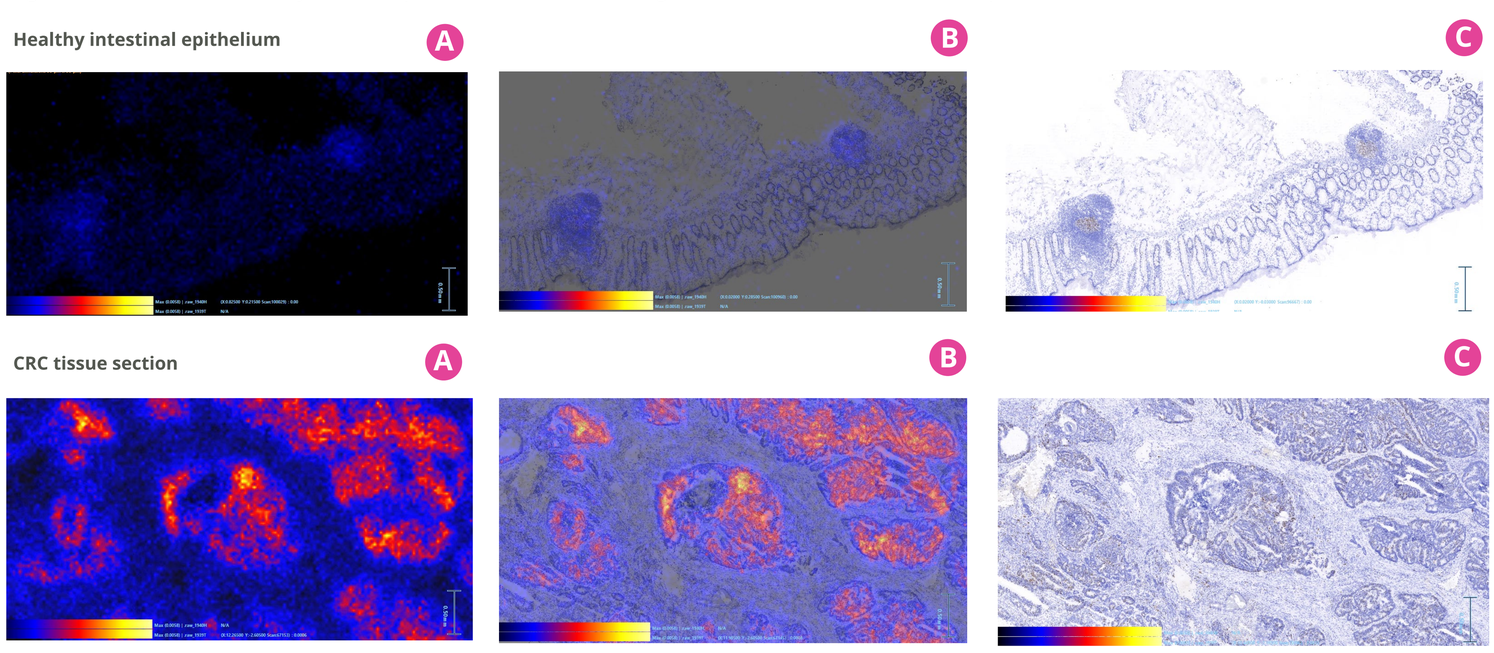

Figure 6. Spatial distribution of putative phosphatidylcholine PC16:0/16:1. (a) Representative ion image of *m/z* 770.5026 ([M+K]⁺) detected in the healthy intestinal epithelium and CRC tissue section. The intensities of the *m/z* 770.5026 are normalized to the same scale and represent a relative quantitative relation. (b) Overlay of the ion image with the IHC image and (c) IHC image after the MSI experiment.

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