USING SPATIAL MULTI-OMICS TO INVESTIGATE THE CONTRIBUTION OF TUMOR MICROENVIRONMENT TO MINIMAL RESIDUAL DISEASE AND INTRINSIC CHEMORESISTANCE OF HIGH-GRADE SEROUS OVARIAN CANCER

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Introduction

Understanding how the abundance, localization, and functional orientation of stromal cells in the tumor microenvironment influences high-grade serous ovarian cancer (HGSC) malignancy and patient survival remains largely unknown. Biomarkers to predict the development of minimal residual disease (MRD) or mechanisms of chemoresistance following primary treatment are currently unavailable. Spatial multi-omics can reveal insights into the tumor immune microenvironment (TIME). We combined multimodal mass spectrometry imaging (MSI) with multiplexed immunofluorescence (mIF), and histology to investigate the cellular and molecular heterogeneity and mechanisms of intrinsic chemoresistance of HGSC.

Experimental Data Collection

Samples were collected from previously untreated HGSC patients undergoing primary cytoreductive surgery, and subsequently classified as MRD+ or MRD- following second look laparoscopy. After FFPE processing and histological assessment, 8 samples (4 MRD+, 4 MRD-) were selected for analysis. Serial sections for were collected from all blocks.

mIF was conducted on a COMET (Lunaphore) utilising a 22-plex antibody panel. This panel was used for cell phenotyping of 29 different cell populations using hierarchical prior knowledge. Figure 1 outlines the multimodal-MSI approach, in which MSI-measured sections were analysed sequentially for metabolites, glycans, and peptides [1]. For metabolite analysis, 1,5-diaminonaphthalene (DAN) matrix was used. For N-glycan imaging, sections underwent PNGase-F digestion, and were sprayed with α-cyano-4-hydroxycinnamic acid (CHCA) matrix. For peptide analysis, on-tissue tryptic digestion was performed followed by coating with CHCA matrix. All matrix and enzyme application were carried out with an HTX M5 Robotic Reagent Sprayer (HTX Technologies). All MSI measurements were conducted on a timsTOF fleX (Bruker). Afterwards, all sections used for MSI were H&E stained and digitized.

An advanced integration pipeline was used to create a common coordinate system and match readouts across the measurement stack. This accounted for the different sections and spatial resolutions of the assays, enabling integrated analysis of the MSI and mIF data, including spatial correlation between analytes across assays, multi-omics tissue segmentation and differential expression analysis.

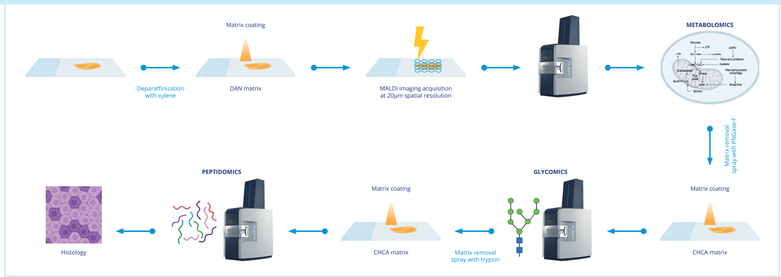

Fig. 1: Schematic diagram summarizing the multiplexed mass spectrometry imaging workflow. After deparaffinisation, sections were directly coated with DAN matrix for metabolite analysis. After metabolite imaging, the matrix was removed with 100% ethanol, then coated with PNGase-F enzyme and incubated in a humidity chamber for in-situ N-glycan release. After PNGase-F digestion, the sections were coated with CHCA matrix. After glycan imaging, matrix was again removed with ethanol, coated with trypsin, incubated in a humidity chamber, and again coated with CHCA. After tryptic peptide image acquisition, matrix was removed using ethanol and the sections were H&E stained using standard protocols.

Results

Reading direction

Joint Visualization of spatial omics HGSC datasets in Weave software

Weave, a web-based spatial multi-omics visualization software package, enabled direct visual comparison of analytes and interactive browsing of data analysis results. Figure 2 is an example showing the measurements and associated data analysis results for one of the HGSC samples. In the current view, the Spatial panel displays the mIF result showing DAPI (blue), PanCK(green), and Ki67(pink) markers highlighting tumor and non-tumor areas, directly compared with cell annotations in the segmented bitmask. The cell annotations in the segmented bitmask are shown in the Cell Groupings panel. The Expression by Cell Set panel shows the expression of glycan Hex5dHex1HexNAc4 (m/z 1809.6416) across the different cell types. It is also possible to view the distribution of individual metabolites, glycans and peptides in the spatial panel, and the H&E staining. Visualization of the different datasets is controlled via the Spatial Layers panel.

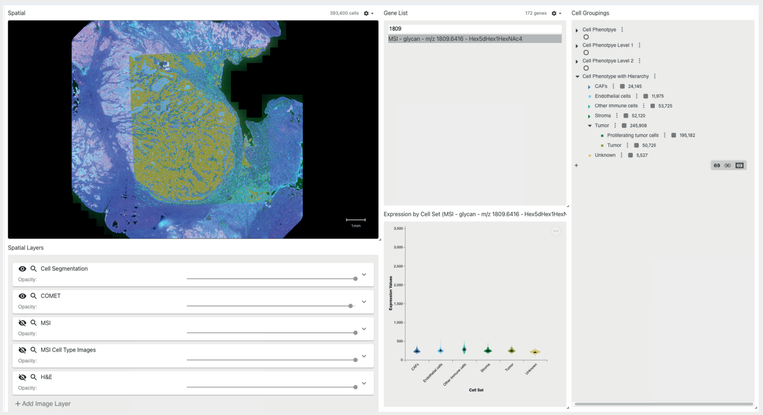

Fig. 2: Screenshot of an interactive report for the single-sample (M1) containing full resolution COMET data, COMET cell segmentation, glycan MSI, peptide MSI. metabolite MSI, and aggregate features such as COMET signal per cell and metabolite signal per cell. In the current figure, an overlay of COMET fluorescence intensity images and MSI glycan (Hex5dHex1HexNAc4) aggregated to cells is shown.

Correlating molecular readouts

The consolidated data structure created after co-registrations allows comparison across different modalities. Figure 3 shows the mean correlations and anti-correlations between a subset of glycan and tryptic peptides in MRD+ samples, allowing us to find molecules detected by different sample preparations and in separate coordinate systems.

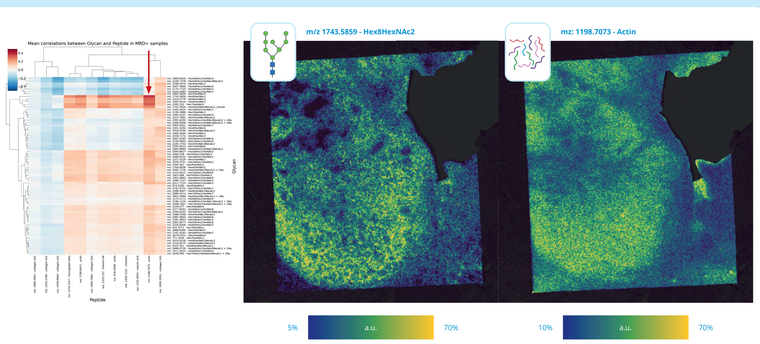

Fig. 3: Correlation matrix between registered multi-omic MSI data. With this method, we identified the glycan Hex8HexNAc2 having a strong correlation with Actin peptide also detected by MSI. a.u. = arbitrary units.

Differential MSI signals by cell type aggregation

Aggregation of cell type information, as defined by the mIF data, allows us to look for tryptic peptide, glycan, and/or metabolite signals that are differentially expressed in specific cell types. For example, glycan Hex5dHex1HexNAc4NeuAc2 + 2Na (m/z 2369.8306) is differentially expressed in CAFs vs tumor regions.

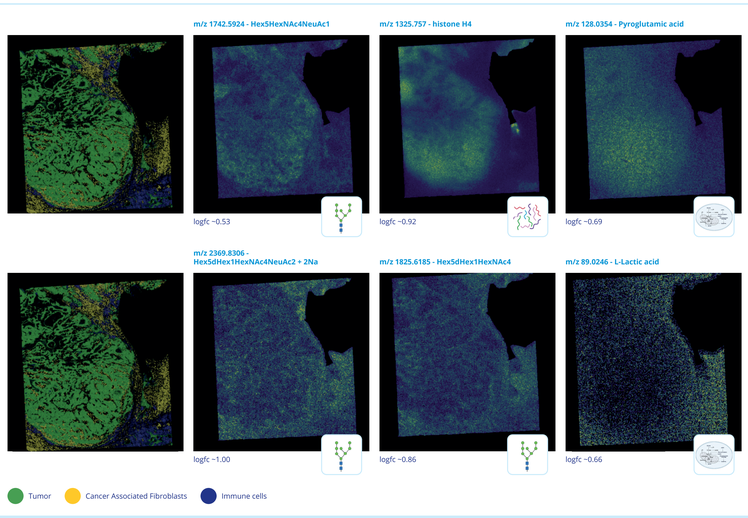

Fig. 4: Automatic means to identify differential cross modality analytes across particular cell types. Tumor cells, CAFs and immune cells identified from COMET were used to find specific glycans, peptides, and metabolites with a differential spatial distribution between tumor and CAFs.

Spatial distance based exploration of Tumor-Stroma interface

Spatial distance analysis of mIF data allowed us to visually and quantitatively explore the tumor stroma interface by computing cell type density as a function of distance to the interface in both the direction of the bulk tumor and the surrounding stroma. Certain cell types like FAP+ CAFs were present at higher density in the stroma very close to the tumor compared to more distal areas.

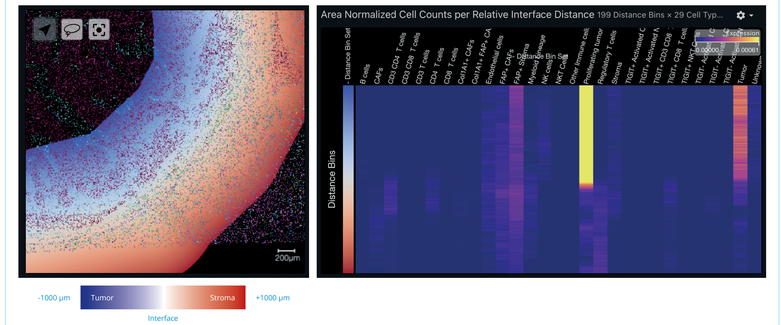

Fig. 5: Spatial analysis on the left showing the interface of tumor and the bulk of the tumor in one sample. Right shows a heatmap where cell type density is computed in 5 micron bins within the interface region.

Conclusion

- We demonstrate a multimodal data acquisition workflow for combined spatial multi-omics of high-grade serous ovarian cancer samples.
- We describe tooling and a cross modality data structure for correlative, differential, and spatial multimodal data analysis.
- Using cell phenotype information, we found interesting preliminary MS markers.
- We preliminary describe the tumor microenvironment with spatial analysis of tumor interface using both cell type and MSI data.

References
[1] Ferri-Borgogno S., Burks J., et al., Cancers 2024 - doi.org/10.3390/cancers16050846