Tumor heterogeneity of glioblastoma analyzed via SpatialOMx and HiPLEX-IHC MALDI Imaging

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Introduction

Glioblastoma (GBM) is the most common and a highly malignant primary brain tumor, with five-year survival rates below 10%. GBMs are characterized by high levels of heterogeneity within the tumor cells and the tumor microenvironment (TME), which consists of blood vessels and necrotic cells intermingled with differentiated and stem-like tumor cells. Tumor heterogeneity influences chemotherapy resistance and local cancer cell dissemination, both connected to a high recurrence rate of >90%. Disruption in metabolism has been reported in GBM, however incorporation of the spatial context that allows assessment of the intratumor heterogeneity and TME have been studied minimally. Here we show a SpatialOMx workflow starting with 4D LC-MS/MS lipidomics. The results were then used for improved annotation for the subsequent MALDI Imaging experiment. The second MALDI Imaging experiment on the ***same tissue*** utilized the MALDI HiPLEX-IHC workflow to detect several different intact proteins on tissue and overlay this information for biochemical correlation.

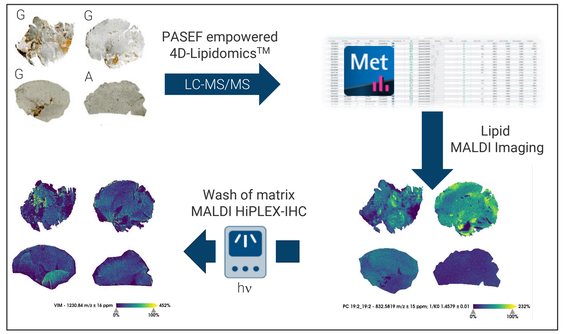

Figure 1. Workflow schematic, starting with the different tissue types, G are GBM samples and A are astrocytoma samples. First a homogenate of each tissue was generated and 4D Lipidomics was performed. Results were used for annotation of a lipid MALDI Imaging run. Afterwards matrix was removed, and staining performed and a subsequent MALDI HiPLEX-IHC imaging run was performed on the same tissue section.

Methods

Fresh frozen tissue sections from three GBM patients and one astrocytoma patient were first homogenized, and lipids were extracted via tert-methyl butyl ether (MTBE) phase separation and measured via LC-MS/MS on a timsTOF fleX. Afterwards the tissue was measured via MALDI Imaging and TIMS on, first acquiring information from lipids, followed by the antibody based MALDI HiPLEX-IHC[1] workflow. Images were acquired on a timsTOF fleX MALDI-2 with 20 µm spatial resolution with TIMS ion mobility on for the lipid imaging. Metaboscape® was used for annotation of MALDI Imaging data using m/z values in combination with the measured collisional cross sections from the 4D Lipidomics experiment. MALDI HiPLEX-IHC was performed according to the AmberGen staining protocol with antibodies specific for tumor (GFAP, Vimentin, Ki67), immune cells, tumor microenvironment (CD68, CD4), as well as a general cell structural marker (Collagen 1A1).

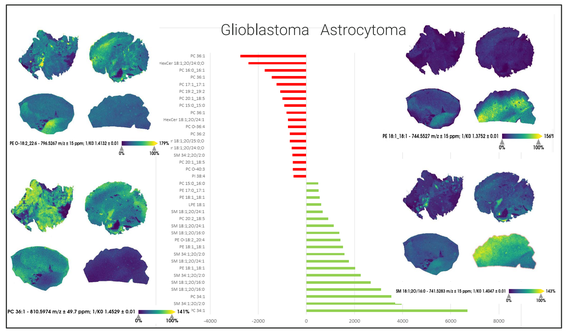

Figure 2. Lipids upregulated in glioblastoma (red) and in astrocytoma (green). RMS normalized peak intensities subtracted with control intensities were used.

Results

- About 300 lipids were annotated and example lipids are displayed that showed a higher expression in glioblastoma or astrocytoma (Fig.2)
- MALDI HiPLEX-IHC imaging based on cell type specific antibodies revealed different cellular compositions in the four brain tissue samples.
- Glia cells were more evenly distributed over the tissue and showed variation between the four samples compared to immune cells.
- CD4 T-cells were more intense in the tumor tissue areas, while macrophages partially colocalized with collagen 1 in round structures, which we hypothesize to be areas of stem cells.
- One glioblastoma tumor contained many B-cells, which are rarely studied in this tumor type.
- Some lipids and metabolites were co-localized with macrophages, B-cells and astrocytes indicating cell-type specific molecular compositions.

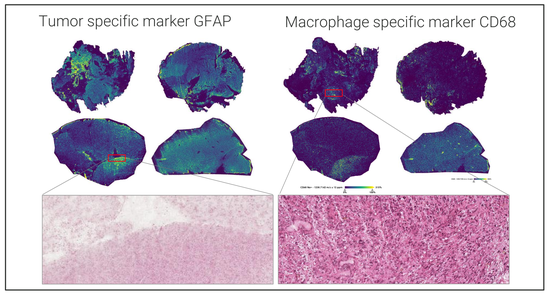

Fig. 3 Two targeted protein markers, GFAP (left) and CD 68 (right) are shown to visualize the specific staining. Zoomed in H&E stains confirm the presence of tumor and immune cells in stained areas

Lipid MALDI Imaging showed spatial heterogeneity in the tumor microenvironment as well as inside the tumor areas. Different phosphatdylcholines (PCs) were tumor specific sphingomyelins showed higher intensities in astrocytes Detailed information on up and down regulated lipids is shown in Fig.2. The specificity of HiPLEX-IHC antibodies was confirmed by H&E staining (see Fig.3).

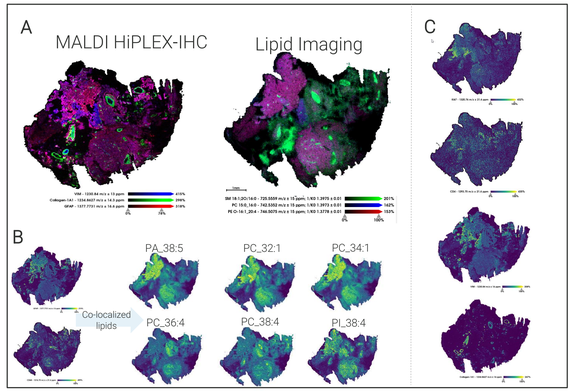

Fig. 4 Overlayed image of three MALDI HiPLEX-IHC markers in comparison to three lipid markers which showed the same distribution. MALDI HiPLEX-IHC data can be integrated in the same SCiLS lab file as the MALDI Imaging lipid data to combine the data and co-localize different lipids to specific markers, as shown for GFAP, CD68, and Collagen 1A1

Summary

The described SpatialOMx® workflow in combination with MALDI HiPLEX-IHC allows an in-depth upfront 4D Lipidomics analysis with confident annotation of spatial resolved lipids in MALDI Imaging. Additionally, the lipid distribution can be co-localized with protein distribution like tumor or immune cell specific markers due to the subsequent HiPLEX experiment on the same slide. Therefore, the up- or down-regulation of lipids, for example PC 36:1 or PC 34:1, can give new insights into the role of lipid metabolism in tumor progression, therapy response or immune cell defense. Furthermore, the PC-derived lipid mediators in cellular communication between cancer and immune cells within the TME, is an essential driver of tumor progression and should be investigated further.[2] Other lipid classes as phosphatidylethanolamine (PE) or hexosylceramides (HexCer) showed a clear up-regulation in GBM samples, whereas sphingomyelins (SMs) were down-regulated. Aberrations in sphingolipid metabolism have been implicated in promoting the aggressiveness of glioblastoma tumors.[3] The co-localization of lipids like PCs or phosphatidic acids with the tumor marker GFAP proves the previous published findings.

Conclusion

- SpatialOMx in combination with MALDI HiPLEX-IHC Imaging can demonstrate spatial and cell-type specific lipid analysis
- This methodology overcomes the inherent heterogeneity of bulk tumors and allows for detailed assessment of intratumor heterogeneity
- Both the tumor and the tumor microenvironment are relevant to glioblastoma recurrence and therapeutic intervention success rates.

Imaging: Spatially-Resolved Omics

References:

1) Yagnik G et al., *J Am Soc Mass Spectrom*. 2021 Apr 7;32(4):977-988.

2) Saito RF et al., *Front Immunol.* 2022 Feb 15;13:768606.

3) Khairunnisa AR et al., *Metabolites.* 2022 Dec 16;12(12):1280