MALDI-IHC-Guided In-Depth Spatial Proteomics
Targeted and Untargeted MSI Combined

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Introduction

Recently, MALDI-IHC was published as novel technology, utilizing the strengths of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) and immunohistochemistry (IHC), achieving highly multiplexed, targeted imaging of biomolecules in tissue. This new technique, enabled workflows to target molecules of interest using MALDI-MSI which is usually reserved for standard IHC. In this poster, the utility of targeted MALDI-IHC and its complementarity with untargeted on-tissue bottom-up spatial proteomics is explored using breast cancer tissue. Furthermore, the MALDI-2 effect on MALDI-IHC treated tissue was investigated.

Method

Results

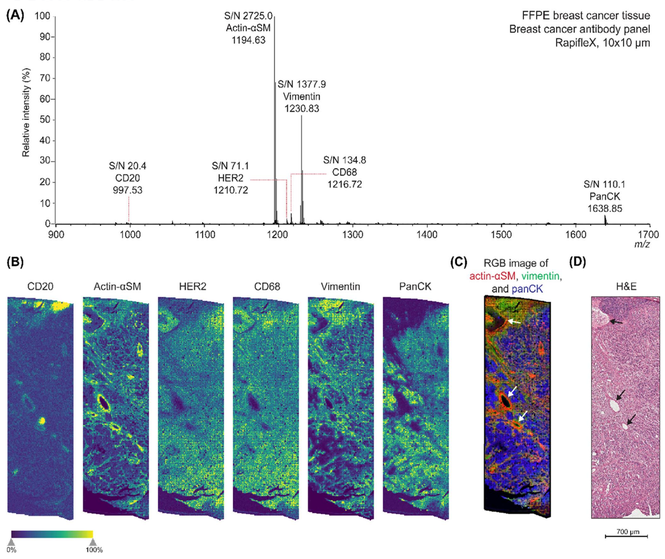

Figure 1. Multiplex MALDI-IHC on the rapifleX for six biomarkers in FFPE human breast cancer tissue (patient 1). (A) Average spectrum of the MALDI-IHC measurement with the breast cancer antibody panel. All six peptide mass reporters were detected. The spectrum was TIC normalized and baseline-corrected. (B) Single-ion images of the mass reporters detected, which were CD20, actin-αSM, HER2, CD68, vimentin, and panCK, respectively. (C) RGB image of actin-αSM (red), vimentin (green), and panCK (blue). (D) H&E image of a consecutive section, showing similar structures as imaged with the MALDI-IHC. White (C) and black (D) arrows point out the vascular lining of blood vessels in the tissue section, which is also highlighted by actin-αSM (B, C).

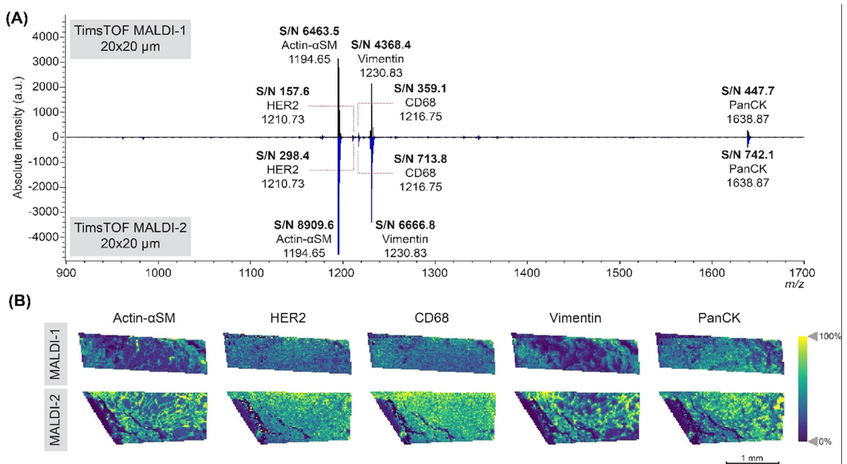

Figure 2. TimsTOF MALDI-1 vs MALDI-2 on breast cancer tissue samples with the PC-MTs. (A) Spectra of timsTOF MALDI-1 (top, black) and timsTOF MALDI-2 (bottom, blue). The S/N of the mass-tags was increased in the MALDI-2 measurement compared to the MALDI-1 data. (B) Single-ion images of five peptide mass reporters detected.

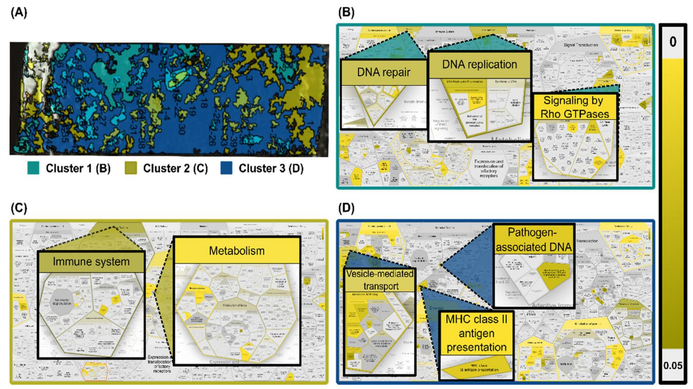

Figure 3. Cluster-specific pathway analysis of over-represented proteins from LMD LC-MS of 0.5 mm2 tissue. (A) Overview of intensity clusters based on MALDI-IHC images. (B–D) Highlighted pathways correlating with over-represented proteins identified exclusively in cluster 1 to cluster 3, respectively. Scale bar = statistical significance of each hit pathway for the sample reflected in color gradient (p-value).

Conclusions

Numerous peptides could be tentatively assigned to proteins, based on untargeted on-tissue digestion and image guided LC-MS of which three proteins were also part of the antibody panel (vimentin, keratins, and actin). Post-ionization with MALDI-2 showed an increased intensity of the PC-MTs and suggests options for the development of new mass-tags. Although the on-tissue digestion covered a wider range of proteins, the MALDI-IHC allowed for easy and straightforward detection of proteins that were not detected in untargeted approaches. The combination of the multiplexed MALDI-IHC with image-guided proteomics showed great potential to further investigate diseases by providing complementary information from the same tissue section and without the need for customized instrumentation.

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