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Multiomic analysis of hepatocellular carcinoma using MALDI Imaging Mass Spectrometry

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Introduction

- The triple helical region (THR) of fibrillar collagens display post-translational modification (PTM) conversion of proline to hydroxyproline (HYP), which can alter cell signaling, recruitment, proliferation, and migration status
- Multiomics analysis (targeted whole protein, glycans, and collagenase digested pepetides) provide extensive information about the tumor microenvironment and cancer progression

Methods

- Heterogeneous human liver hepatocellular carcinoma (HCC) FFPE tissue sections with multiomic analysis of glycans, collagen peptides, and whole targeted proteins
- Optimized procedures for enzyme application and MALDI HiPLEX-IHC methods

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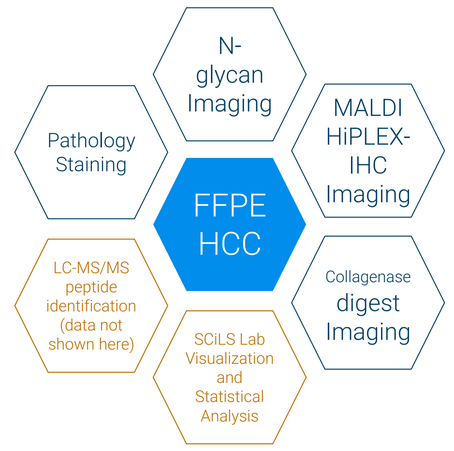


Figure 1. Analysis Methods for HCC tissue

Key Results

- Full visualization of heterogeneous TME is possible with multiple histological stainings, the MALDI HiPLEX-IHC workflow, N-glycan imaging, and collagen peptides.
- Collagen peptides are further enhanced with MALDI-2 capabilities.

Figure 2. MALDI HiPLEX-IHC imaging shows HCC heterogeneity.

Vimentin- red; poor prognosis, recurrence, metastasis

Collagen α1(1) – blue

Cd11b- myeloid marker – green.

Right panel = Positive ion mass spectrum of photocleavable markers.

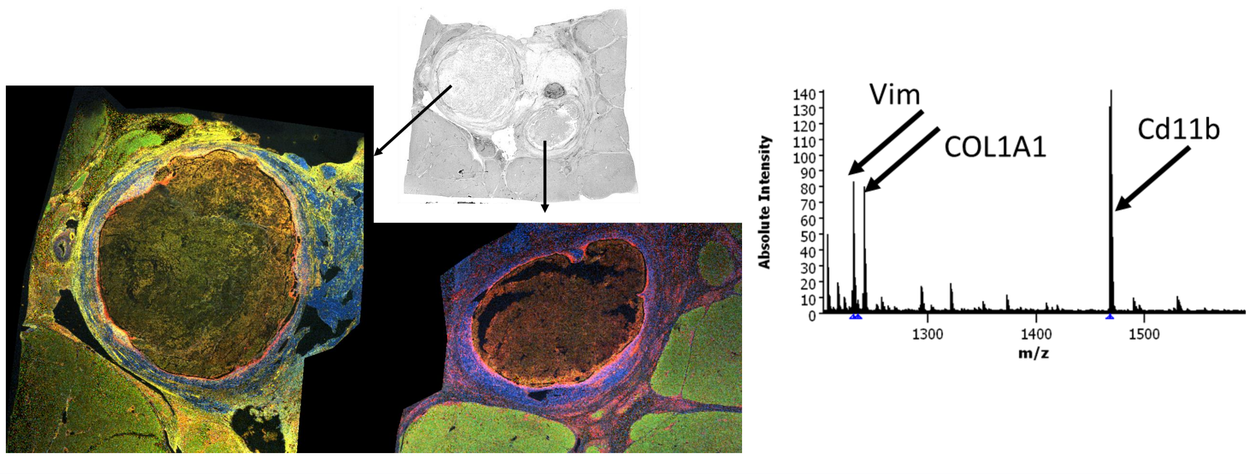
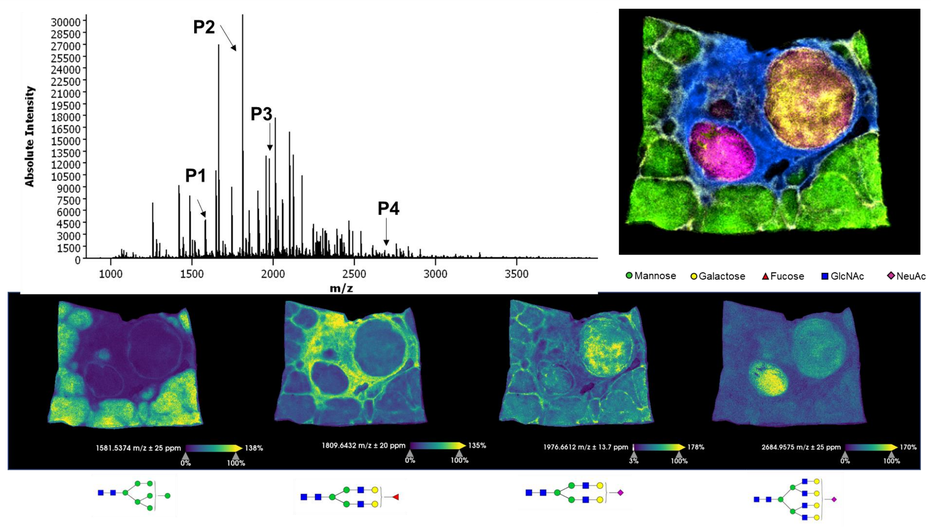
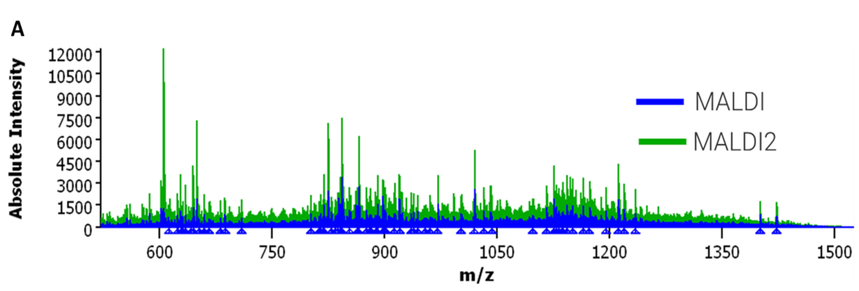


Figure 3. MALDI N-Glycan imaging displays unique glycan localization in hepatocellular carcinoma.

Upper left panel; m/z chromatogram, upper right; overlayed MSI of P1, P2, P3 and P4, lower panel: individual MSI and respective glycan structure assignment.





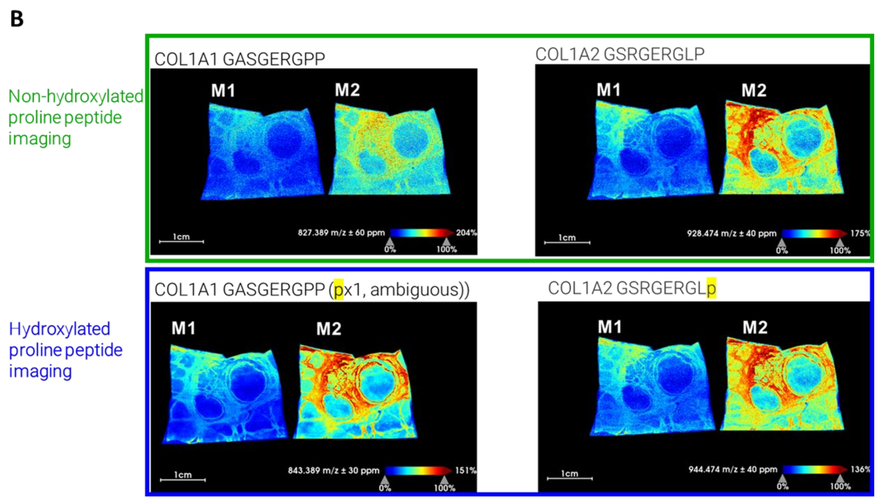


Figure 5. MALDI-2 enhances imaging of collagenase peptides. (A) MALDI-2 improves enhances nonpolar peptide ionization with increased S/N. (B) MALDI-2 enhancement of both hydroxylated and non-hydroxylated proline collagenase peptides

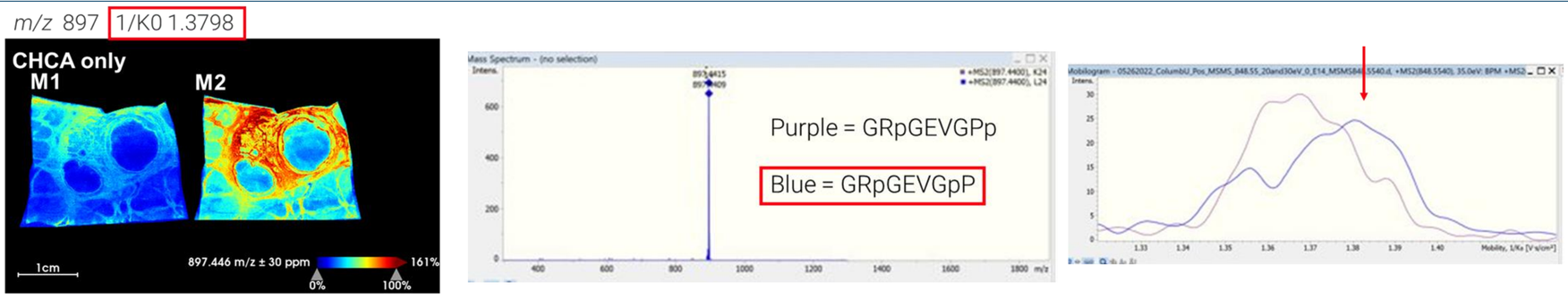


Figure 6. MALDI-2 and ion mobility discern positional isomers. Positional isomers displaying identical m/z values are represented in the m/z image (left) and mass chromatogram (middle) but display unique 1/K0 values in the mobilogram (right).

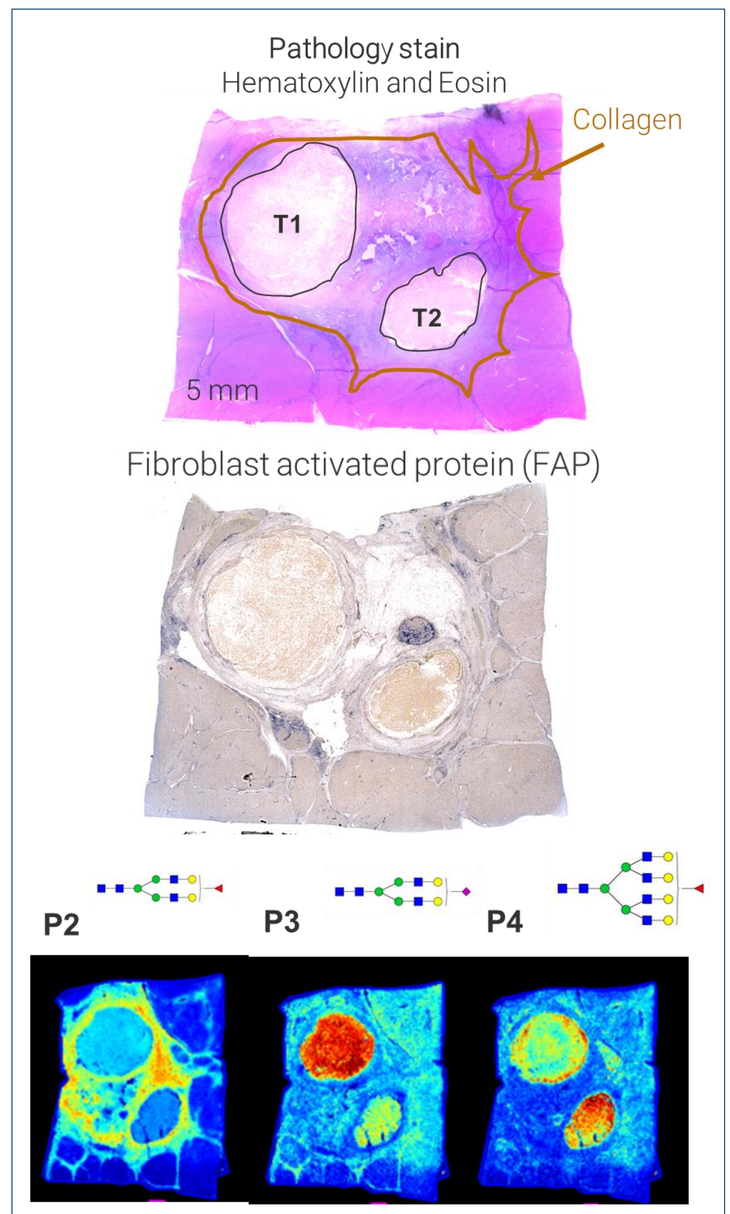


Figure 4. MALDI N-Glycan imaging. Smaller tumor stains high in fibroblast activated protein, marker for poor outcome (top and middle). MALDI N-glycan imaging analysis shows heterogenous tumors embedded within collagenous fiber around tumors (lower)..

- The combination of MALDI-2 and TIMS separation enhances detection and identification of nonpolar collagenous peptides from clinical specimens including positional isomers.
- The multiomic approach provides a comprehensive picture of tumor biology.
- The addition of targeted protein imaging is a valuable tool to study tumor biology.

Summary