MP 734 Quantitative spatial proteomics combined with lipidomic analysis of human hippocampus using laser capture microdissected cells from MALDI-imaged tissue sections

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Introduction

To compare the proteomic and lipidomic signatures of hippocampal trisynaptic cell-types (HI-cells), specifically the dentate gyrus, CA1 and CA3 regions, we are combining laser capture microdissection (LCM) with MALDI imaging (MSI) and micro-scale tandem mass tag (TMT) proteomics analysis. Proteins are analyzed from the same slide, directly after matrix-assisted laser desorption/ionization (MALDI) imaging analysis, thereby facilitating co-registration of identified lipids. This way, the results are not confounded by analyzing cell populations from two different tissue sections. We report distinct cell-type enriched molecular networks within heterogeneous tissue microenvironments of the hippocampal formation as an archetype for other human brain regions.

Methods

Human hippocampus was cryosectioned (10uM) using uncoated or poly-L-lysine-coated ITO-slides (3 replicates total). MALDI-imaging lipid analysis was performed on Bruker Rapiflex MALDI TOF/TOF in triplicate. HI-cells were excised using a Zeiss LCM (~1e5um ^{2} with ~1500 cells) and the boundaries were overlaid with the original MALDI imaging data using SCILS Lab software. The phenol fraction of Trizol extracted proteins were processed using a modified single pot extraction method (SP3), and labeled with isobaric mass tags using a modified TMTpro protocol. Peptides from four basic reverse phase fractions were analyzed with 120 min nanoflow reverse-phase chromatography gradient on a Thermo Orbitrap Fusion Lumos interfaced with Easy nLC 1200. Tandem MS2 spectra were processed by Proteome Discoverer (v2.5) and analyzed with Mascot (v.2.7).

Preliminary Data

We have demonstrated that the same section can be used for MALDI imaging, laser capture microdissection, and proteomics. The poly-L-lysing coating had no impact on any of these experiments.Positive ion mode MALDI imaging of human brain sections revealed a large number of phospholipids associated with the dentate gyrus, CA1, and CA3. Region of interest (ROI) analysis of MALDI imaging data showed more similarities in phospholipids between CA1 and CA3 than either CA1 or CA3 and the dentate gyrus. Using tandem MS directly from tissue, we have begun identifying specific lipid m/z peaks that are unique to each region, as well as m/z's which are found in all three regions of the trisynaptic circuit.Using approximately 1500 cells per ROI with an estimated 6 ug combined total protein, which were obtained by laser-capture microdissection from the MALDI-imaged slides, we identified and quantified 3,411

proteins from 20,328 peptides in 49,030 spectra at 5% false discovery rate. on the grouped abundances, each of the three different ROIs contained substantially different protein profiles. Using a 20% fold change cut off, the dentate gyrus displayed 509 up-regulated protein with a p-value of $\leq$0.05 when compared to CA1, and 309 up-regulated proteins compared to CA3. The dentate gyrus granular cell layer is expected to have significantly different protein expression as it contains many smaller and densely packed cells than the CA1 and CA3 regions. We now can directly link the proteomic differences with the distinct lipid profile differences as determined by MALDI imaging from the same cell populations in human brain sections across three distinct brain regions.

Novel Aspect

Direct comparison of proteomic and lipidomic profiles of the same hippocampal cell populations in a single human brain section.

Conflict of Interest Disclosure

The authors declare no competing financial interest.