Study of whole cell proteome- the proteomic difference of astrocyte between mouse brain cortical and hippocampal regions

Chien-Chang Huang, Weng Man Chong, Chiung-Yun Chang, Hsiao-Jen Chang, Jung-Chi Liao* , Syncell Inc., Taipei, Taiwan
For more information, please visit https://syncell.com/ * corresponding author (Email: jcliao@syncell.com)

Introduction

Astrocytes are a type of glial cells within the central nervous system. They participate in neurogenesis, synaptogenesis, regulation of blood–brain barrier permeability, and maintaining extracellular homeostasis. Notably, different populations of astrocytes located in specific brain regions show unique morphological and functional traits; however, the proteomic differences among these region-specific astrocytes remain poorly understood. In this study, we used optoproteomics, an advanced technology combining microscopy-guided automated photo-biotinylation, purification, and mass spectrometry, to isolate and identify proteins in cortical and hippocampal astrocytes from the mouse brain, aiming to find proteins unique to these two brain regions. Astrocytes in mouse brain cryosections were immunostained with an antibody against glial fibrillary acidic protein (GFAP) and targeted with two-photon illumination, which effectively activated proprietary photosensitive biotin-based probes in the medium, triggering high-precision protein biotinylation. This cycle operated automatically across thousands of fields of view, ensuring enough biotinylation of proteins for subsequent pulldown and proteomic analysis via LC-MS/MS. Known astrocyte markers, including GFAP, LAMC3, and PARK7, were identified in the proteomes. Comparative analysis revealed several candidates enriched specifically in either the cortex or hippocampus, some of which have not been reported before; these were further validated through immunofluorescence. The results showed that at least PLEKHB1 and SYBP3 colocalized with GFAP in cortical astrocytes but were nearly absent in hippocampal astrocytes. In contrast, the hippocampal-enriched candidate MINK1 was confirmed in hippocampal astrocytes and absent in cortical astrocytes. Overall, optoproteomics enables the discovery of new, region-specific astrocyte markers and generates testable hypotheses for further functional studies of these regionally unique proteins.

Microscoop® : A Novel Platform for Spatial Proteomic Profiling via Machine Learning-Guided Microscopic Photolabeling

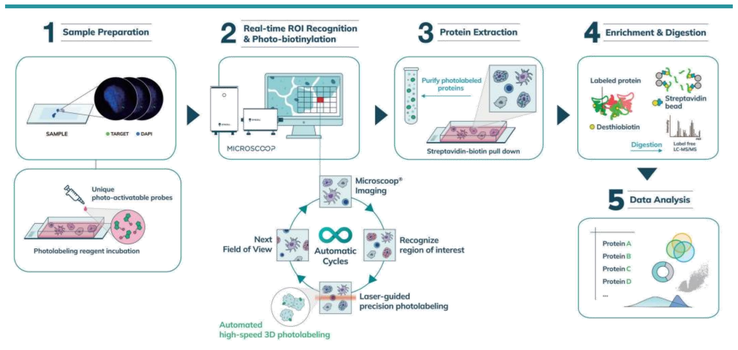
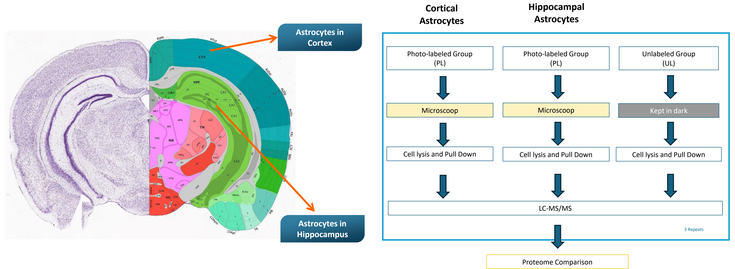


Fig. 1 |
STEP 1. Regions of interest (ROIs) in cells or tissue samples were marked by immuno-fluorescence staining. Fluorescence images were acquired and segmented using conventional image processing steps.
STEP 2. Samples were incubated with photoactivatable biotin probes. Photolabeling at subcellular ROIs were performed with two-photon illumination of the Microscoop® system.
STEP 3. Biotinylated proteins were purified by affinity pulldown and analyzed by LC-MS/MS.
STEP 4. Hypothesis-free protein biomarkers were identified with proteomic analysis.

Single Cell Proteomic Discovery : Comparing the Proteome of Astrocytes in the Cortex and Hippocampus of Mouse Cerebrum



Precise Biotinylation of Astrocytes in Mouse Brain Regions

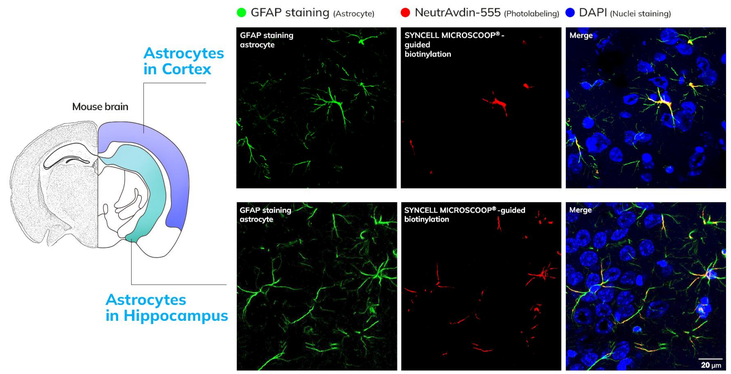


Fig. 3 | Astrocytes were stained with GFAP (glial fibrillary acidic protein) antibody in coronal sections of the C57BL/6 mouse brain (Green). The tissue sections were blocked using Synlight-Rich™ kits, then incubated with a photo-activatable probe. A modified Otsu thresholding method was first applied to identify GFAP-positive astrocytes for photolabeling. Photolabeling was then performed on approximately 3000 astrocytes per cortex or hippocampus region with Microscoop® to ensure the collection of enough biotinylated proteins for mass spectrometry analysis. Confocal imaging confirmed the accuracy of biotinylated photolabeling on astrocytes (Red). Merged images indicate precise labeling. Note that the morphology of astrocytes varies significantly between cortical and hippocampal regions.

Known Astrocyte Proteins Identified by Microscoop®

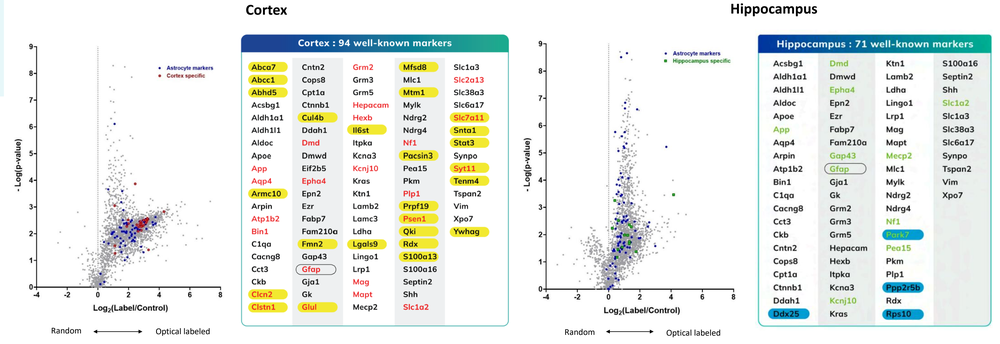
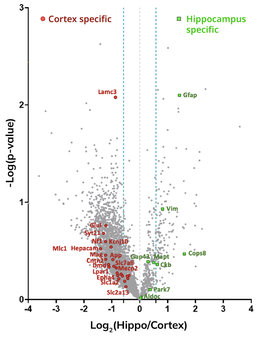


Fig. 4 | Microscoop® photolabeled biotinylation enhances protein isolation from cortical and hippocampal astrocytes. The spatial proteomic results demonstrated that, in comparison with 352 known astrocyte markers, 94 corresponded in the cortical region (27%) and 71 in the hippocampal region (20%). In the volcano plot of the cortical region, well-established astrocyte markers (blue) and cortical-associated astrocyte markers previously reported in the literature (red) were identified, with yellow highlights indicating proteins specifically enriched in cortical astrocytes. In the volcano plot of the hippocampal region, well-established astrocyte markers (blue) and hippocampal-associated astrocyte markers previously reported (green) were identified, with blue highlights indicating proteins specifically enriched in hippocampal astrocytes. The findings suggest that Microscoop® technology effectively facilitates the analysis of proteomic differences between cortical and hippocampal astrocytes.

Top 20 Abundant Region-Specific Protein Candidates

Gene	Description
LAMC3	Laminin subunit gamma-3
CST3	Cystatin-C
PLEKHB1	Pleckstrin homology domain-containing family B member 1
FBLN5	Fibulin-5
FERMT2	Fermitin family homolog 2
RAB21	Ras-related protein Rab-21
GLTP	Glycolipid transfer protein
ADAM23	Disintegrin and metalloproteinase domain-containing protein 23
ADAM22	Disintegrin and metalloproteinase domain-containing protein 22
SEPTIN4	Septin-4
STXBP3	Syntaxin-binding protein 3
C1QC	Complement C1q subcomponent subunit C
CNTN2	Contactin-2
LGI3	Leucine-rich repeat LGI family member 3
CD9	CD9 antigen
NTM	Neurotrimin
HSD17B12	Very-long-chain 3-oxoacyl-CoA reductase
NCAM2	Neural cell adhesion molecule 2
LAMB1	Laminin subunit beta-1
BCAM	Basal cell adhesion molecule



Gene	Description
COTL1	Coactosin-like protein
CDC42	Cell division control protein 42 homolog
Slc6a11	Sodium- and chloride-dependent GABA transporter 3
Rpl8	Large ribosomal subunit protein uL2
PSD3	PH and SEC7 domain-containing protein 3
MACROH2A2	Core histone macro-H2A.2
CORO1C	Coronin-1C
Slc2a3	Solute carrier family 2, facilitated glucose transporter member 3
Dstn	Destrin
MINK1	Misshapen-like kinase 1
Ap2s1	AP-2 complex subunit sigma
Hras	GTPase HRas
Mapre3	Microtubule-associated protein RP/EB family member 3
Fabp3	Fatty acid-binding protein, heart
Akr1a1	Aldo-keto reductase family 1 member A1
MSN	Moesin
Park7	Parkinson disease protein 7
Txn	Thioredoxin
Ass1	Argininosuccinate synthase
Slc1a2	Excitatory amino acid transporter 2

Fig. 5 | Comparative analysis of cortical and hippocampal proteomic datasets, volcano plots based on fold change ratios revealed distinct clustering of region-specific markers. From this analysis, we identified the top 20 novel candidate proteins, a subset of which were selected for antibody validation using immunostaining.

Validation of Region-Specific Protein Candidates in Cortical and Hippocampal Astrocytes

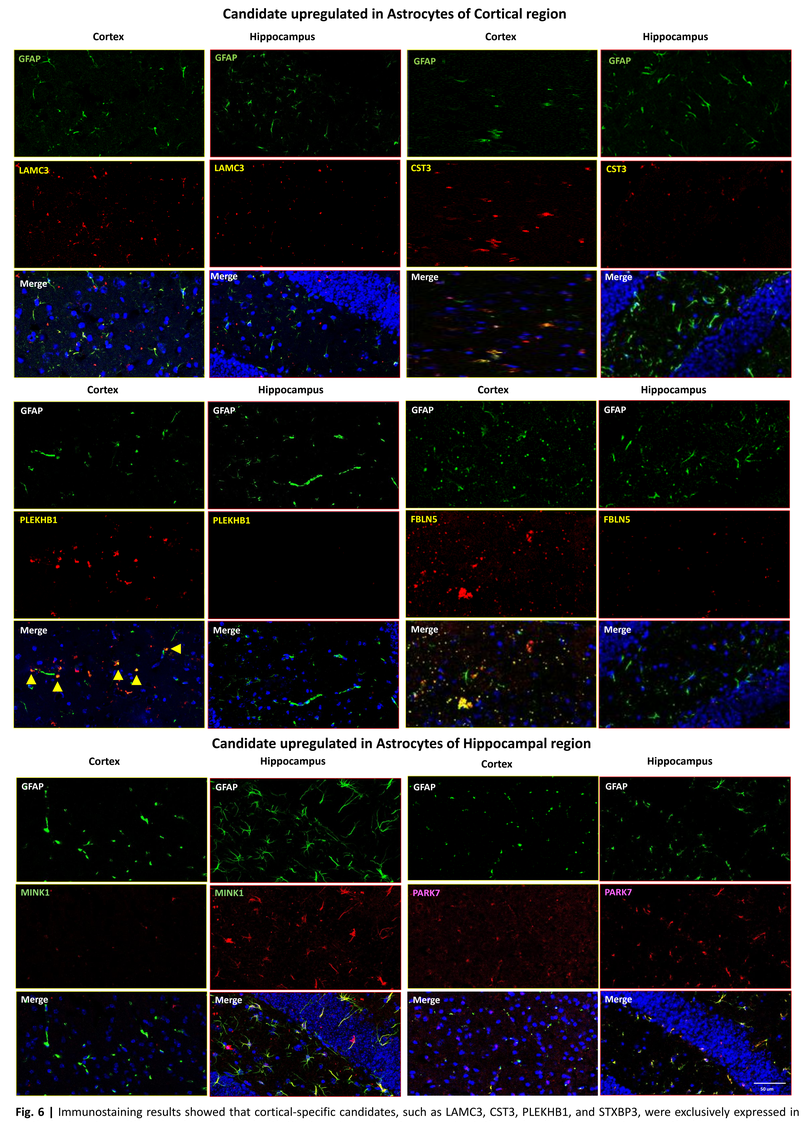


Fig. 6 | Immunostaining results showed that cortical-specific candidates, such as LAMC3, CST3, PLEKHB1, and STXBP3, were exclusively expressed in cortical astrocytes, with IF signals co-localized with GFAP-positive astrocytes. In contrast, this co-localization was not seen in the hippocampal region. Conversely, hippocampal-specific candidates, including MINK1 and PARK7, were selectively expressed in hippocampal astrocytes, with antibody signals overlapping with GFAP staining primarily in hippocampus-localized astrocytes and not in cortical astrocytes. These imaging results confirm that the Microscoop® platform successfully identified novel proteins specific to cortical and hippocampal astrocytes, respectively.

Discussion

This study employed the Microscoop® technology to investigate single-cell proteomics, focusing on astrocytes as the target cell type. The main goal was to characterize proteomic differences across different regions of the mouse brain, enabling more detailed analysis of single-cell differentiation. We successfully identified unique proteomic profiles between cortical and hippocampal astrocytes and confirmed the spatial distribution of selected candidate proteins through fluorescence staining, showing co-localization with GFAP-positive astrocytes. These results validate the regional specificity of the proteins identified. Looking ahead, the use of the Microscoop® platform offers great potential for discovering additional functional proteins, which could improve the accuracy of both diagnostic and therapeutic strategies.