Highly multiplexed spatial single-cell multiomic imaging of tau neuropathology in human brain tissue sections

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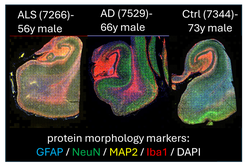
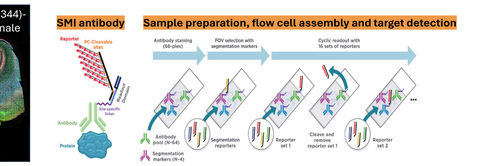
Introduction

- Intraneuronal deposition of aberrant tau protein into neurofibrillary tangles (NFTs) closely correlates with neurodegeneration and cognitive decline. Pathogenic tau induces cell death in some neurons while other neurons evade apoptosis by entering cellular senescence, a complex stress response leading to a change in cell fate[1, 2]. Removing senescent cells improves brain structure and function underscoring the importance of exploring senescent cells, their consequences across the brain and potential molecular targets for interventions[1, 3, 4].
- To study the transition of post-mitotic neurons into senescence, along with its impact on neighboring cells and tissue-wide effects, high-resolution spatial analyses are essential[5, 6]. These analyses should capture changes in protein levels, post-translational modifications (e.g., phosphorylation), and gene expression. A spatial molecular imager (SMI) provides ultra-high plex detection of RNA and protein, enabling detailed molecular profiling within a spatial context.
- Here we showcase the multi-omic capabilities of the SMI in detecting the entire transcriptome and >68 proteins from healthy and diseased human brain sections.



68 plex human protein panel

Human WTx RNA panel (18936 plex): covers nearly all biology



CosMx™ SMI Protein & RNA Workflows

Protein data collection

7529= AD with neuritic plaques

SMI antibody

Sample preparation, flow cell assembly and target detection

RNA data collection

SMI ISH probe

Sample preparation, flow cell assembly and target detection

3D Image analysis to localize RNA transcripts or proteins (RNA example shown)

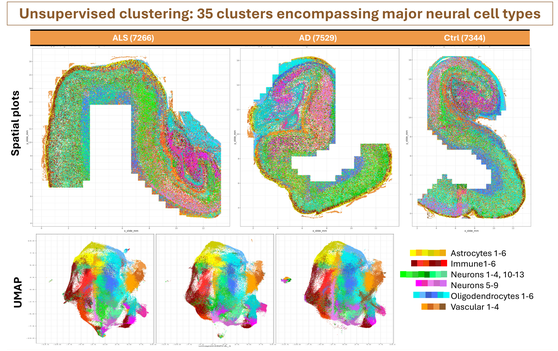
Assign proteins or RNA to single cells based on morphology-based segmentation

A new cell segmentation framework leverages high-plex protein images to improve protein run segmentation

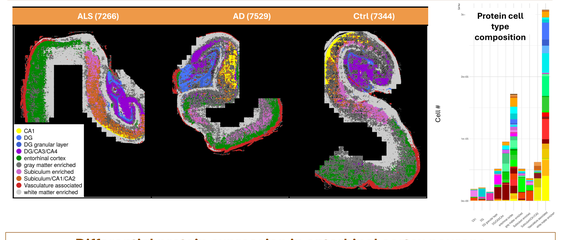
The SMI assay detects protein and RNA targets with oligonucleotide barcode-conjugated antibodies and barcoded RNA probes via several rounds of reporter binding and fluorescence imaging. Cells are segmented based on protein stains and decoded RNA targets or proteins are assigned to individual cells.

High-plex spatially resolved protein data enables analyses of pathogenic tau and its effects on neighboring cells

Unsupervised clustering: 35 clusters encompassing major neural cell types



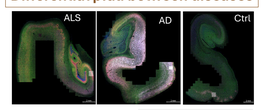
Clustering on neighborhood cell type composition identified tissue niches matching anatomical structures and enabling DE analyses across regions



Differential protein expression in entorhinal cortex neurons

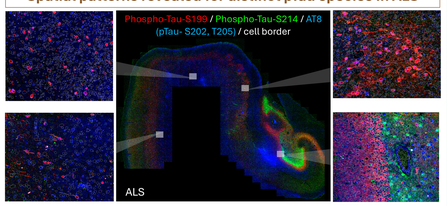
Left: The control tissue expresses higher TH (tyrosine hydroxylase) protein levels than disease tissues, which has implication in dopaminergic neurotransmitter activities. **Right:** The AD tissue expresses higher levels of tau phosphorylated at Ser214 & Ser396, consistent with higher pathogenic tau and the observed neurofibrillary tangle (NFT) pathology.

Differential pTau between diseases



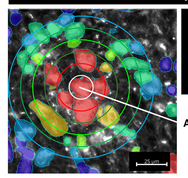
Some tau epitopes are phosphorylated in ALS in distinct spatial locations. Tau is phosphorylated at many epitopes and in many regions in AD.

Spatial patterns revealed for distinct pTau species in ALS

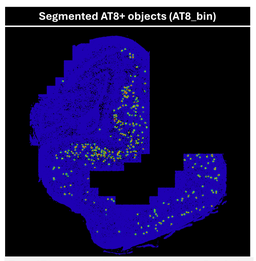


Protein object analyses: AT8 segmentation and differential expression at distances from NFTs

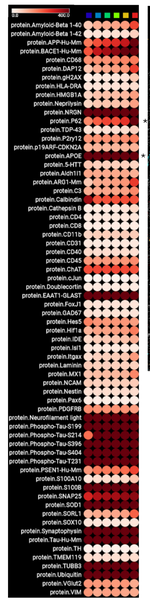
Distance bins from AT8+ objects

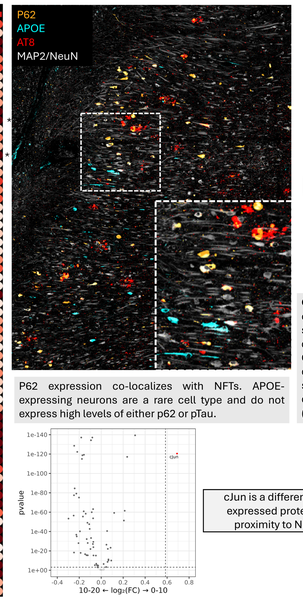


Segmented AT8+ objects (AT8_bin)



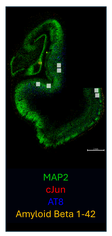
Top: AT8 was used to segment NFTs. Proximity bins were created surrounding NFTs and spatially mapped (**bottom**) across the AD brain. **Right:** Neuronal protein expression differs based on their spatial association to NFTs. For example, amyloid processing machinery (Aβ 1-40, APP, BACE1 and Neprilysin) and p62 increase as cells approach NFTs while calbindin uniquely decreases in neurons that are in closer proximity to NFTs.





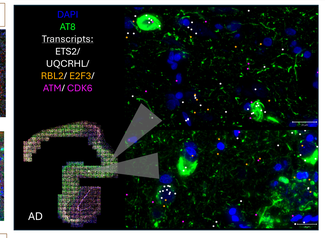
P62 expression co-localizes with NFTs. APOE-expressing neurons are a rare cell type and do not express high levels of either p62 or pTau.

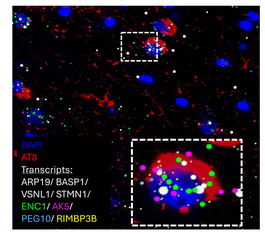
cJun is a differentially expressed protein in proximity to NFTs



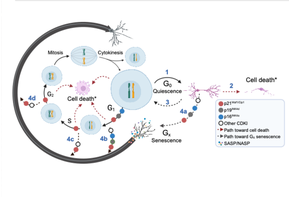
c-Jun is involved in the progression of the G1 phase of the cell cycle among other functions. Left: Overlay of MAP2 (neurons, green), cJun (red), AT8 (NFTs, blue) and Aβ 1-42 (orange) across the AD brain. **Right:** Increased cJun protein expression in AD neurons close to AT8+ NFTs.

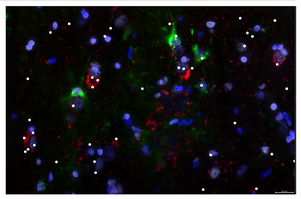
SMI protein and RNA data reveal G_X neurescence



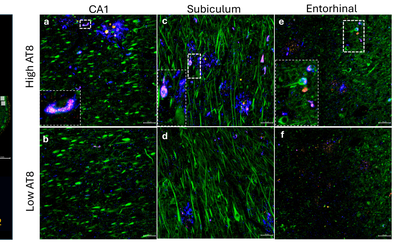


Previously discovered neurescent genes spatially map and colocalize with NFTs. Left. Previous studies developed senescence eigengenes to identify senescent cells in single nucleus RNA sequencing datasets[2]. The eigengenes indicated that NFTs-bearing neurons were a primary source of senescent cells in the AD brain. The genes within the eigengene shown to contribute most weight to the senescence phenotype included *ETS2, RBL2, E2F3, ATM* and *CDK6*. CosMx SMI indicate that these genes are upregulated in NFT-bearing neurons. Further differential expression analyses identified a novel gene, *UQCRHL*, that also spatially maps and co-localizes with NFTs (green). **Right.** Differential gene expression analyses of CosMx SMI datasets has revealed additional, novel, putative neuronal senescence, "neurescence" genes.





Cell cycle events and cell fate options of postmitotic neurons. Left: Neurons differentiate from precursor cells, exit the cell cycle and may 1) remain in G_0 through effective cell maintenance, damage repair and suppressing cell cycle events; 2) undergo cell death; 3) re-engage the cell cycle where they may undergo cell death, continue through various stages of the cell cycle including G_1 and S or; 4) enter senescence (G_X). Entry to G_X may occur directly from G_0 (4a) or by exiting the cell cycle after stress-induced re-entry (4b-4d). CDKI: cyclin dependent kinase inhibitor; SASP: senescence associated secretory phenotype; NASP: neurescence associated secretory phenotype; *Cell death: multiple neuronal cell death mechanisms, beyond apoptosis, have been described for neurodegenerative diseases. **Right:** Neurons with NFTs (red) co-express the G_X gene, *CDKN2D* (white dots), which encodes $p19^{INK4d}$. Green: GFAP (astrocytes); Blue: DNA (nuclei): Gray: Histone (nuclei).



Whole transcriptome RNA data identifies spatially colocalized gene modules that differentiate disease from control tissue

Unsupervised clustering identified 22 clusters, encompassing major neural cell types

UMAP visualization showing 22 distinct clusters representing major neural cell types from tissues affected by amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and control tissues. Control tissues are from two different ages. Young: 36 years-old and Old: 73 years-old. Clusters reflect similarities and differences in cell type profiles across disease states and age groups. Spatial plots depict the tissue distribution of the cell types identified in the UMAP analysis, showing their physical locations within the tissue sections.

InSituCor identifies spatially colocalized gene modules that differ with disease

InSituCor detects gene expression patterns that are spatially related, while filtering out trivial correlations, such as those that might occur simply due to cell type similarities. Applying InSituCor to the CosMx SMI dataset revealed multiple gene modules that were unique to (a) ALS entorhinal cortex; (b) young control brain; (c-d) AD brain with either elevated as shown in (c) or decreased expression as shown in (d); (e) control; and (f) ALS brain.

Summary

CosMx SMI Platform Capabilities:

- 50nm resolution of analytes across entire FFPE tissue sections
- Spatially-resolved multiomic data including whole transcriptome + 68-plex protein, and post-translational modifications
- Identification of major brain cell types and spatial niches
- Whole transcriptome analyses reveals spatially resolved, co-localized gene modules
- Protein object analyses reveals molecular, cellular and niche changes in relation to neuropathology

Preliminary Findings and On-Going Analyses:

- Molecular signatures of distinct neuropathologies (NFTs and Aβ plaques)
- Spatial resolution and intersection of neuropathology and neurescent cells
- Multi-analyte, cell and niche specific changes and vulnerabilities to distinct diseases (ALS vs AD)

References

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Funding to MEO: Department of Veterans Affairs I01BX005717, NIA R01AG068293, Cure Alzheimer's Fund, AFAR/Hevolution, Rainwater Charitable Foundation).