Spatial Proteomic Analysis of Alzheimer's Disease Human Brain using Multiplexed Imaging

Poster # 10429

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Introduction & Aim

Alzheimer's disease (AD) is a genetic and sporadic neurodegenerative disease and a common cause of cognitive impairment acquired in midlife and late life. AD is pathologically defined by the presence of β-amyloid-containing plaques and phosphorylated tau containing neurofibrillary tangles. Effective therapies for the treatment and/or prevention of AD are lacking. The examination of the histopathological features of AD may reveal cellular relationships that contribute to disease etiology leading to potential novel therapeutic strategies. The Cell DIVE Multiplexed Imaging Solution, in combination with IF/IHC-validated antibodies from Cell Signaling Technology (CST), can be used to computationally examine synaptic processes and spatially and molecularly define cells, such as glia and neurons, surrounding pathological hallmarks in AD. Segmentation and clustering analysis can identify spatially co-localized populations of cells, including subpopulations of microglia defined by specific disease-associated microglia markers. CST's broad portfolio of validated antibodies enables the detection of altered synaptic processes and cell-type populations in the context of human disease tissue. Here we demonstrate multiplexed Cell DIVE imaging using a novel CST® panel to probe AD brain. We examine the protein landscape in diseased tissue in the context of Amyloid-β (Aβ) and tau expression. The ability of cell-type specific markers, combined with multiplexed tissue imaging will provide a new approach for the neuroscience research community to understand spatial heterogeneity of the human brain and their contribution to disease.

Results

- The spatial profiling of AD brain reveals a significant increase in phosphorylated tau containing neurofibrillary tangles and β-amyloid containing plaques, indicative of neurodegeneration. Such structures are more abundant around the outer periphery of the AD brain tissue. While cell types like astrocytes marked by GFAP+ cells demonstrate clustered and heterogeneous expression in the periphery of AD brain tissue, they are more enriched and homogenously expressed in the inner core of the brain tissue **(Figure 1A-C)**.
- Additionally, the β-amyloid+ plaques in the Alzheimer's brain demonstrated a wide distribution of sizes in terms of cross-sectional area compared to area occupied by the neuronal cell types **(Figure 1D-E)**.
- There is a significant loss of various cell phenotypes in Alzheimer's brain, for instance, microglia marked by TMEM119+ cells, and astroglia marked by GFAP+ cells and S100B+ cells. Interestingly, in addition to the Alzheimer's-associated Tau markers, IBA1+ cells are increased in Alzheimer's brain compared to the control. **(Figure 1F)**.
- Although there is a global reduction in various astrocytes and microglia cell types in Alzheimer's brain, there is an accumulation of astrocytes and microglia (GFAP+, S100B+, TMEM+ and IBA1+ cells) close to relatively larger plaques, potentially indicative of astrogliosis and microgliosis around those regions of the brain **(Figure 2A-D)**.

Table 1. Study Design: Antibodies and Tissues

Target	Clone	Conjugate	Concentration	Dilution
Myelin Basic protein	D8X4Q	AF488	200 µg/mL	1:100
AQP4 (D1F8E)	D1F8E	AF488	200 µg/mL	1:100
Iba1/AIF-1	E404W	AF555	200 µg/mL	1:100
p-Tau (T217)	E9Y4S	AF555	200 µg/mL	1:100
TMEM119	E3E4T	AF555	200 µg/mL	1:100
Enolase2 (E2H9X)	E2H9X	AF555	200 µg/mL	1:100
GFAP	GA5	AF647	200 µg/mL	1:100
beta-Amyloid	D3D2N	AF647	200 µg/mL	1:100
PSD95	D74D3	AF647	200 µg/mL	1:100
p-Tau (T181)	D9F4G	AF647	200 µg/mL	1:100
p-Tau (T217)	E9Y4S	AF750	200 µg/mL	1:100
S100B	E7C3A	AF750	200 µg/mL	1:100
Tau (GT-38)	GT-38	AF750	200 µg/mL	1:100
TMEM119	E3E4T	AF750	200 µg/mL	1:100
ApoE (pan)	E8C2U	AF750	200 µg/mL	1:100

Slide	Tissue	Catalogue Number
AB-0054	Human Adult Normal: Brain	T2234035
AB-0055	Alzheimer's Disease: Brain	T2236035Alz
AB-0057	Alzheimer's Disease: Brain: Amygdala	T2236036Alz

Methods & Materials

CST antibodies undergo a vigorous validation process to ensure antibody performance on FFPE tissue. All antibodies in this study were direct conjugates (**Table 1**). Following preliminary validation, conjugated antibody solutions with the optimum degree of labeling and concentration were randomly assigned to a round, without optimization and used for subsequent staining of normal and Alzheimer disease brain tissue. Tissue was obtained from a commercial source (BioChain; **Table 1**). Slides were imaged on the Cell DIVE imager using four channels plus DAPI, with automatic AF removal, corrections, and stitching. Imaging rounds were conducted over a 2-week period. At round 5 slides were stored for long term at 4°C for future experiments. Fully stitched images were imported, fused, and analyzed using AIVIA 13. Using AI-driven analysis, the expression of markers (shown in **Table 1**) was quantified for the segmented phenotypes and used for subsequent analyses (**Figure 1C**).

Conclusion

Iterative staining and imaging of human adult brain tissues with CST antibodies and Cell DIVE multiplexed imaging solution enabled spatial characterization of AD human brain. Cell DIVE multiplexing solution is tissue preserving, enabling further probing to comprehensively characterize the AD-associated markers within the neuronal environment. Further comprehensive studies focused on specific sections of the brain including the amygdala (**Figure 3**) can reveal novel spatial characteristics associated with the disease.

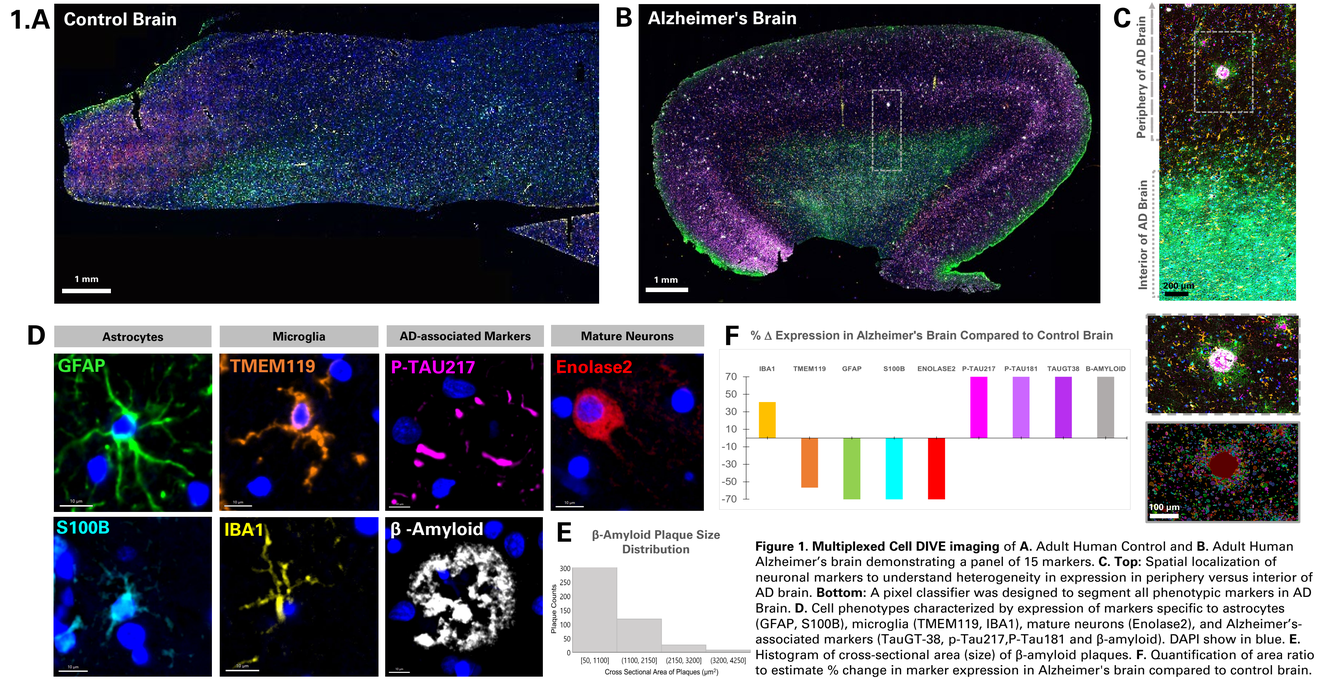

Figure 1. Multiplexed Cell DIVE imaging of **A.** Adult Human Control and **B.** Adult Human Alzheimer's brain demonstrating a panel of 15 markers. **C. Top:** Spatial localization of neuronal markers to understand heterogeneity in expression in periphery versus interior of AD brain. **Bottom:** A pixel classifier was designed to segment all phenotypic markers in AD Brain. **D.** Cell phenotypes characterized by expression of markers specific to astrocytes (GFAP, S100B), microglia (TMEM119, IBA1), mature neurons (Enolase2), and Alzheimer's-associated markers (TauGT-38, p-Tau217,P-Tau181 and β-amyloid). DAPI show in blue. **E.** Histogram of cross-sectional area (size) of β-amyloid plaques. **F.** Quantification of area ratio to estimate % change in marker expression in Alzheimer's brain compared to control brain.

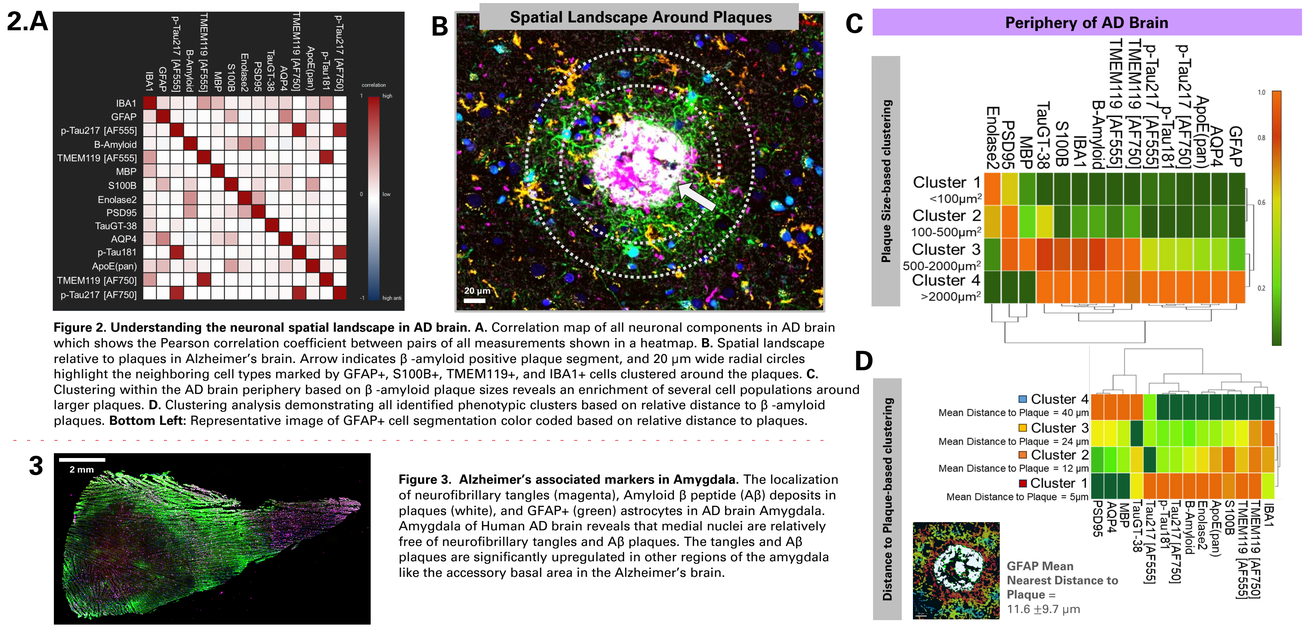

Figure 2. Understanding the neuronal spatial landscape in AD brain. A. Correlation map of all neuronal components in AD brain which shows the Pearson correlation coefficient between pairs of all measurements shown in a heatmap. **B.** Spatial landscape relative to plaques in Alzheimer's brain. Arrow indicates β -amyloid positive plaque segment, and 20 µm wide radial circles highlight the neighboring cell types marked by GFAP+, S100B+, TMEM119+, and IBA1+ cells clustered around the plaques. **C.** Clustering within the AD brain periphery based on β -amyloid plaque sizes reveals an enrichment of several cell populations around larger plaques. **D.** Clustering analysis demonstrating all identified phenotypic clusters based on relative distance to β -amyloid plaques. **Bottom Left:** Representative image of GFAP+ cell segmentation color coded based on relative distance to plaques.

Figure 3. Alzheimer's associated markers in Amygdala. The localization of neurofibrillary tangles (magenta), Amyloid β peptide (Aβ) deposits in plaques (white), and GFAP+ (green) astrocytes in AD brain Amygdala. Amygdala of Human AD brain reveals that medial nuclei are relatively free of neurofibrillary tangles and Aβ plaques. The tangles and Aβ plaques are significantly upregulated in other regions of the amygdala like the accessory basal area in the Alzheimer's brain.

Questions?
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