Evaluating Alzheimer's disease-associated gene markers with Xenium in situ gene expression

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1. Introduction

Studying cellular and molecular states underlying Alzheimer's disease (AD) is critical to understand disease mechanisms. The novel high throughput Xenium In Situ platform uses a microscopy based read-out and curated, pre-validated gene panels to identify spatial patterns of gene expression with subcellular resolution in either fresh frozen (FF) or formalin-fixed paraffin-embedded (FFPE) tissue sections. Here, we used the Xenium In Situ platform to evaluate gene expression in FFPE brains from an AD transgenic (tg) mouse model (TgCRND8) and its wild-type (WT) counterpart. By including a set of custom add-on genes to the Xenium mouse brain gene panel, we demonstrated the ability to demarcate main brain cell types and activation status to estimate cellular composition and gene expression during health and disease. Additionally, we demonstrated sensitivity, robustness and specificity of the Xenium assay.

2. Experimental overview

We obtained FFPE brain samples from the TgCRND8 mouse model (n=3) and its wild-type littermates (n=3) at three age timepoints, corresponding to progressive Aβ deposition. Xenium offers the capability to add up to 100 custom genes to its base gene panels, allowing researchers to tailor the assay to their scientific needs. Sections were subjected to the Xenium In Situ assay, using a custom panel targeting additional 99 genes. The custom gene panel was designed to include genes associated to activated states of glial cells (microglia and astrocytes) and Aβ-paque inducible genes (PIGs) that were curated from the literature[1, 2, 3, 4].

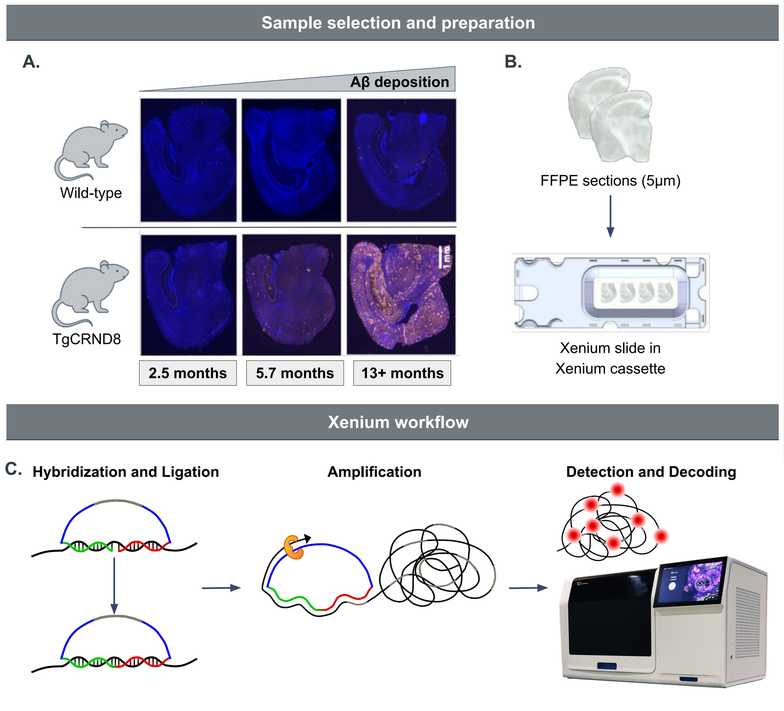

Figure 1. Experimental overview. (A) Immunofluorescence (IF) images showing Aβ deposition in FFPE brain samples obtained from the TgCRND8 mouse model and its WT littermates at three age timepoints, corresponding to mild (2.5 mo), moderate (5.7 mo), and severe (13+ mo) Aβ deposition. (B) Technical duplicates of coronal brain sections (5 µm) were placed on 12 x 24 mm Xenium slides (four sections per slide), subjected to deparaffinization and de-crosslinking and placed in a Xenium cassette in preparation for the Xenium workflow (C) DNA probes were hybridized to mRNA targets (347 total genes). DNA probes have two target binding regions and a gene specific barcode that identifies a target transcript. The target binding regions on probe bind and ligate to form a circular DNA probe, which is amplified by rolling circle amplification. On the instrument, fluorescently labeled detection probes were hybridized to rolling circle products for a total of 15 cycles of in situ imaging to generate a unique optical signature for each gene. An on-instrument analysis pipeline decodes the fluorescent signals. Nuclear boundaries were determined by segmentation of a DAPI image, while cell boundaries were estimated by dilation of the nuclear boundary. Xenium Analyzer on-instrument analysis assigned transcripts to cells by nuclear morphology for downstream visualization in Xenium Explorer and analysis.

References

1. Keren-Shaul H, *et al.*; A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell, 2017
2. Sala Frigerio C, *et al.*; The Major Risk Factors for Alzheimer's Disease: Age, Sex, and Genes Modulate the Microglia Response to Ab Plaques. Cell Reports, 2019
3. Chen WT, *et al.*; Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease. Cell, 2020.
4. Clarke LE, *et al.*; Normal aging induces A1-like astrocyte reactivity. PNAS, 2018.
5. https://satijalab.org/seurat/articles/integration_mapping.html

3. Xenium is sensitive, robust and specific

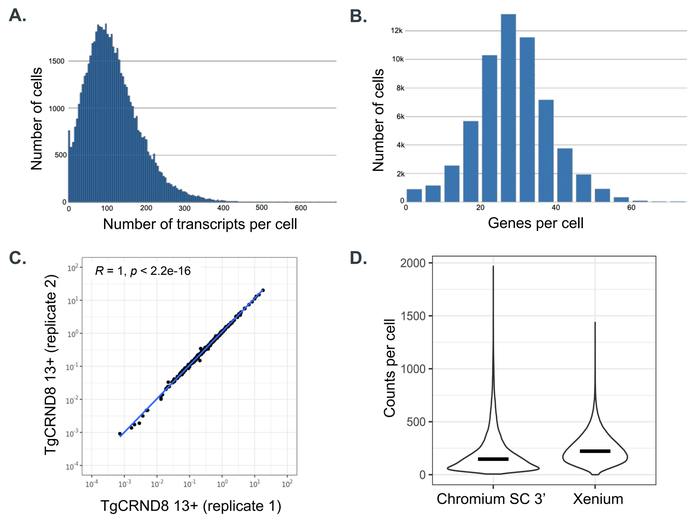

Figure 2. Xenium is sensitive and assay is highly reproducible. (A) Representative plot (TgCRND8 13+ months animal) of transcript counts per cell. Median transcripts per cell across all included replicates = 231 [IQR: 194 - 243]. (B) Representative plot (TgCRND8 13+ months) showing the distribution of number of genes detected per cell. Presence of genes is defined as being >0 transcripts. (C) Representative scatter plot (TgCRND8 13+ months) showing correlation of total transcript counts per gene between technical replicates (Pearson correlation *R* value range = 0.94 - 1, across all replicates). (D) Violin plots showing Xenium sensitivity compared to paired Chromium Single Cell 3' gene expression (SC 3' GEX), which was was down-sampled to the number of genes in the Xenium panel (347) prior to the analysis. Chromium SC 3' GEX data were previously obtained from the same animals using an entire fresh frozen brain hemisphere through the Multiome ATAC + Gene Expression kit.

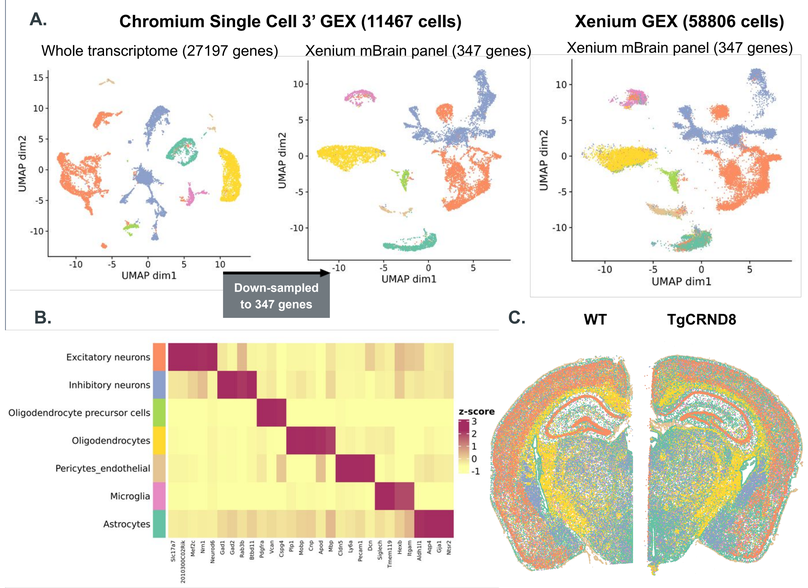

Figure 3. Xenium mouse brain gene panel represents main cell types. (A) Representative UMAP projections showing supervised cell type labeling of a coronal section (WT 13+ months). The reference for cell label transfer into Xenium data comes from the paired Chromium SC 3' V2 GEX data. The down-sampled (347 genes) Chromium SC 3' GEX data and Xenium data for each animal-time point were subjected to expression normalization and clustering. Transfer of cell types labels from Chromium SC 3' GEX data to Xenium data was performed using the label transfer procedure in Seurat[5]. Cells in the Xenium data (median = 57593 [IQR: 56035 - 58930) cells per 5 µm coronal section) were projected onto the Chromium SC 3' GEX UMAP visualization. Labels are colored by super class (e.g. Inhibitory neurons: Blue). (B) Heatmap showing selected cell gene markers from the Xenium mouse brain panel for major brain cell types. (C) Representative spatial distribution of cells in coronal sections of WT (right) and TgCRND8 (left) coronal sections at 13+ months of age.

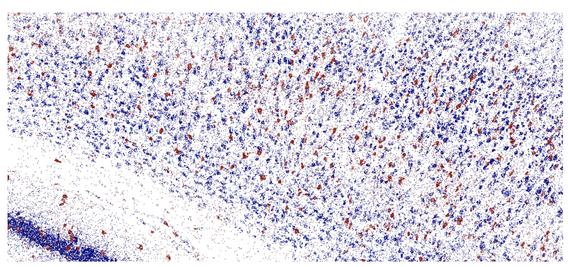

Figure 4. Excitatory and inhibitory neurons highlight marker specificity. Each dot marks one transcript from Glutamatergic markers (**Blue**; Slc24a7, Nrn1, Epha4, 2010300C02Rik, Neurod6) or GABAergic markers (**Red**; Gad1, Gad2, Dner, Btbd11, Cacna2d2) within a primary somatosensory area and CA1 of a coronal section. Scale bar = 200 µm.

4. Estimating cell composition and gene expression between healthy and disease states with Xenium

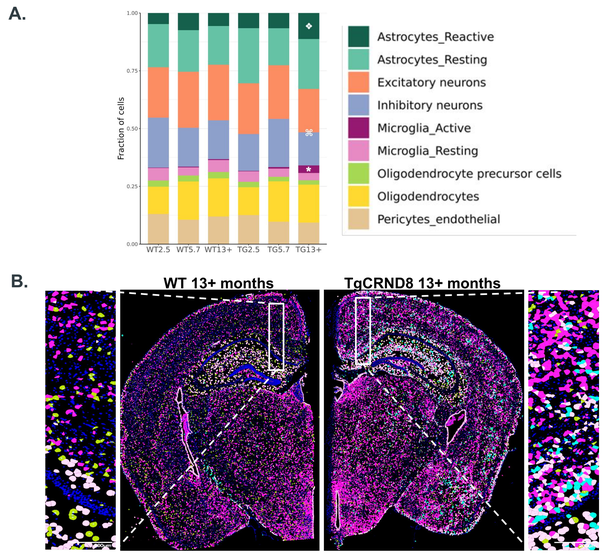

Figure 5. Cellular composition between health and disease states over time. (A) Bar plot showing differences in cell proportions between WT and TgCRND8 mice over time. In TgCRND8, there is a decrease in neuronal cell populations (⌘), and an increase in astrocyte (❖) and microglia (*) populations over time, particularly reactive glial cells, defined based on expression levels of a set of gene markers (reactive astrocytes: *Serpina3n, Vim, Osmr, C4b, C3, Gbp2*; reactive microglia: *C1qa, C1qb, C1qc, Ctsd, Cd63, Apoe, Tyrobp, B2m, CD74*), curated from the literature and included in the custom gene panel. (B) Enrichment of activated glial cells in TgCRND8 mice, compared to WT, at 13+ months of age, was visualized using Xenium Explorer (resting microglia in **olive**, reactive microglia in **lime green**, resting astrocytes in **dark pink**, reactive astrocytes in light pink), nuclei in **blue**. Detail shows areas in the brain cortex (scale = 200 µm).

5. Simultaneous visualization of Aβ-plaques, glial cells and PIG RNA markers

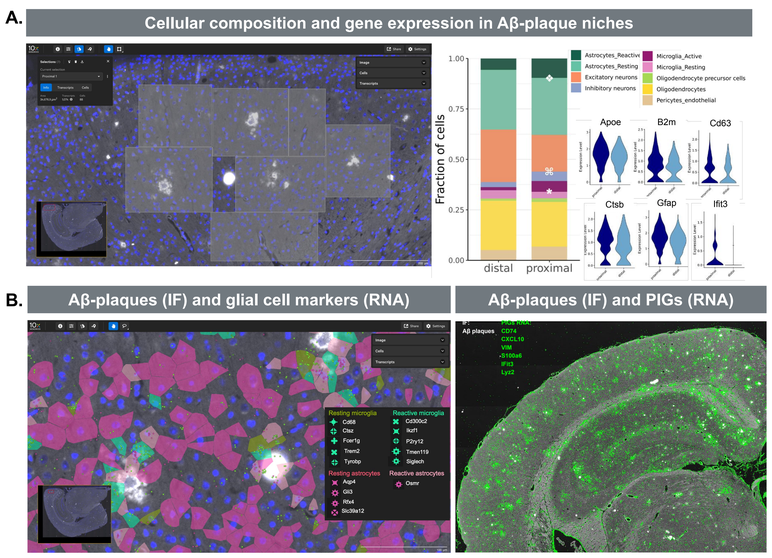

Figure 6. RNA from Xenium and Aβ protein immunofluorescence (IF) from the same tissue section to evaluate cell composition and gene expression in Aβ plaque proximal and distal areas. After 15 cycles of imaging on the Xenium instrument, sections were immunostained for Aβ-plaques (white; 6E1 antibody) using a fluorescent secondary antibody (Alexa-fluor 750) and DAPI (**blue**). Because RNA and protein data were obtained from the same section, the two DAPI images were registered and overlaid with RNA and protein expression. (A) Selection of Aβ-plaque proximal (~50 µm from Aβ-plaques boundaries) and distal regions (at least ~50 µm away from the nearest Aβ-plaque boundary) in selected cortical region of a TgCRND8 13+ month old mouse, using the *lasso* tool in Xenium Explorer (A-left). Bar plot showing cell proportions in selected Aβ-plaque proximal and distal regions (A-right). An increase in gliosis is observed in Aβ-plaque proximal areas, particularly in reactive microglia and astrocytes. Violin plots highlight genes enriched in in Aβ-plaque proximal compared to distal areas (A-bottom right). (B) Aβ-plaques IF (white) are shown registered with resting and reactive microglial and astrocyte cells, and their RNA markers, in Xenium Explorer (B-left), and selected PIGs in cortical region (**lime green**; B-right).

6. Conclusion

- The Xenium in situ assay is sensitive, robust and specific, enabling researchers to estimate disease-induced cellular and gene expression changes in AD transgenic mice.
- Custom genes enabled us to localize glial cellular states in the mouse brain.
- Post-Xenium workflow IF staining of Aβ-plaques in the same coronal sections allowed for registration with Xenium data to study cellular composition and gene expression in the plaque niches.

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