Highest-Plex Single-Cell Spatial Whole Transcriptome and 1000+ Proteome Identify a New Cell Type and Neural State In Alzheimer's Disease Brains

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Abstract: Cellular senescence is a cell state characterized by apoptosis resistance, cell cycle arrest, and proinflammatory secretome. Senescent cells accumulate with aging, and their role in neurodegenerative diseases like Alzheimer's disease (AD) is increasingly recognized. However, senescent cells are challenging to study *in situ* due to their loss of cell identity, heterogeneous and complex cellular and molecular features. Here we used spatial proteogenomics to explore postmortem human AD brains and identified a unique type of neuron, referred to as "neurescent", that is arrested in a senescent-like cell state we refer to as "G_X". We used the Digital Spatial Profiler platform for high-plex multiomic analyses of discrete regions of interest (ROIs) in formalin fixed paraffin embedded tissue sections while preserving spatial context. The assays use antibodies and *in situ* hybridization probes coupled to photocleavable DNA barcodes readout with NGS sequencing. We employed a stackable multi-omics platform including the Human Neural Proteome Atlas (500+ targets), Human Immuno-Oncology Proteome Atlas (570+ targets), an ultra-high-plex neural proteomic panel, and a Whole Transcriptome RNA panel. We examined cellular and molecular changes near and far from AD-associated amyloid and tau pathologies, as well as proteogenomic features of senescent cells, in postmortem hippocampal tissues from AD, age-matched controls, and young healthy controls. We also utilized the CosMx spatial molecular imager (SMI) for ultra-high plex detection of spatially resolved RNA and protein with subcellular resolution. The multiomics approach first detects proteins with oligonucleotide barcode-conjugated antibodies and then exposes sections to protease digestion followed by transcript detection with barcoded RNA probes. The targeted proteins focus on neural cell typing and AD pathology, whereas the RNA portion of the assay provides an unbiased view of 18,000+ protein-coding genes. Lastly, we analyzed serial sections on CellScape, which allowed us to include difficult targets previously identified as markers of senescence, but that are expressed at low levels. Overall, our data provide evidence for a novel neuronal cell state and type, which we refer to as "G_X" arrested neurescent cells, and their spatial proximity to AD neuropathology. With these newly identified molecular features, there is potential to begin investigating their druggability as therapeutic targets and utility as biomarkers for clinical trials aimed at clearing senescent cells in Alzheimer's disease, which are currently in Phase 2 testing.