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Unravelling the Genetic and Transcriptomic Interplay in Microglia Using ResolveOME workflow

Introduction

ResolveOME is a single-cell multiomic platform that enables paired genomic and transcriptomic profiling from the same individual cell[1]. By sequentially separating cytoplasmic RNA from the nucleus, the workflow captures the poly(A) transcriptome while preserving genomic DNA for whole-genome amplification using primary template-directed amplification (PTA). The RNA- and DNA-derived products are then processed independently for sequencing, allowing cell identity and transcriptional state to be linked directly with genomic variation in the same cell. This paired design is particularly valuable for studying somatic mosaicism, where rare mutations can be connected to specific cell types and cellular states without relying on measurements from separate cell populations. ResolveOME therefore provides a framework for integrating single-cell transcriptomic annotation with high-resolution genomic analysis, including somatic mutation burden, mutational signatures, and clonal relationships.

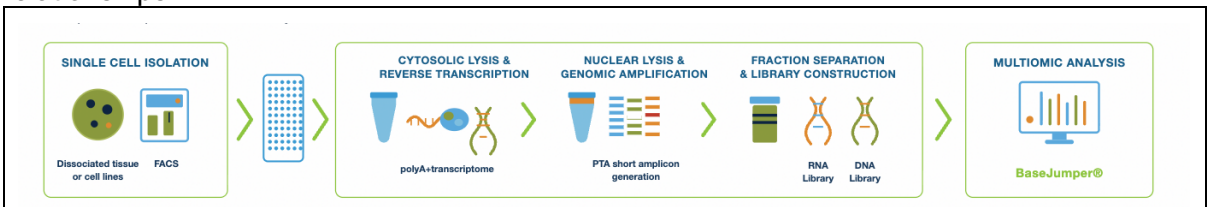


Figure 1. ResolveOME workflow for paired transcriptome and genome profiling from individual cells. Single cells are isolated and arrayed into multiwell plates, followed by sequential cytosolic and nuclear processing. Cytosolic RNA is reverse-transcribed to capture the poly(A) transcriptome, while genomic DNA from the remaining nucleus is amplified using PTA. The RNA and DNA fractions are subsequently separated and converted into sequencing libraries, enabling matched transcriptomic and genomic profiling and integrated multiomic analysis at single-cell resolution. (Image adapted from BioSkryb Genomics)

Experimental design

Post-mortem brain tissue from an individual with Alzheimer's disease was dissociated to generate a single-cell suspension, from which **30 individual cells** were isolated and processed using the ResolveOME workflow. Transcriptomic libraries were first generated and analyzed to determine the cellular identity of each successfully profiled cell based on its RNA-seq expression pattern and reference-based cell-type annotation. Following transcriptomic annotation, cells classified as microglia were prioritized for genomic analysis. **8 microglial cells** were selected, and their corresponding PTA-amplified genomic DNA was subjected to whole-genome sequencing and downstream somatic mutation analysis. This sequential design allowed transcriptomic information to guide cell selection before genomic profiling, thereby enabling somatic mutation patterns to be examined specifically within transcriptionally defined microglia from Alzheimer's disease brain tissue.

Data processing

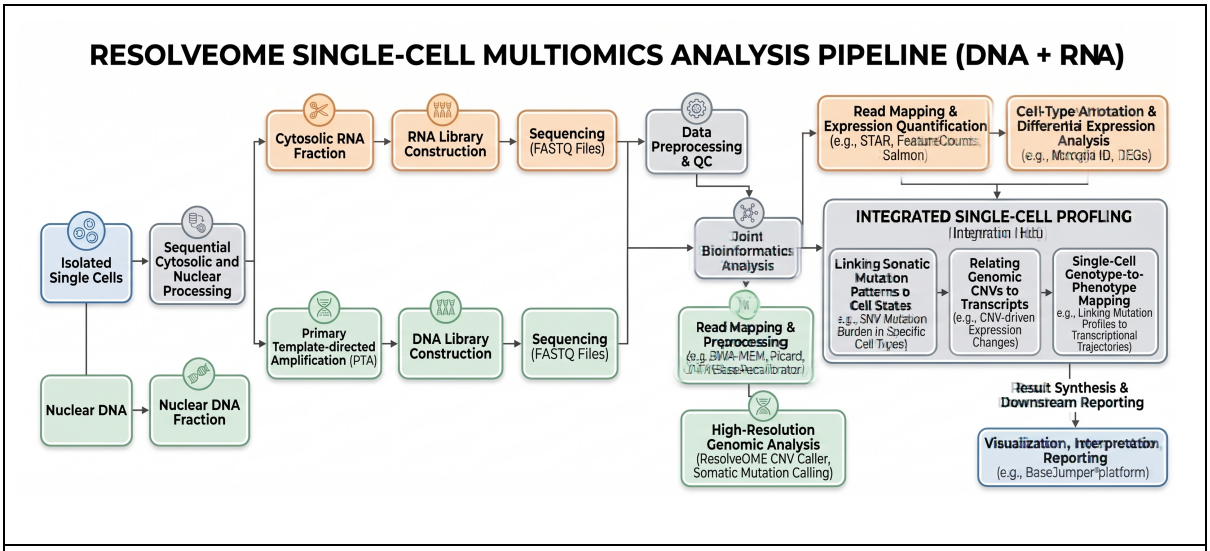


Figure 2. ResolveOME single-cell multiomics workflow for integrated genomic and transcriptomic analysis. Individual cells are isolated and subjected to sequential processing to separate cytosolic RNA from nuclear DNA. Cytosolic RNA is reverse-transcribed and processed for single-cell RNA sequencing (scRNA-seq) to perform read mapping, expression quantification, and cell-type annotation (e.g., identifying specific cell populations like microglia and differential expression analysis). Concurrently, nuclear DNA undergoes Primary Template-directed Amplification (PTA) followed by whole-genome sequencing (WGS) to execute high-resolution genomic calling, including somatic mutation calling (SNVs/Indels) and copy number variation (CNV) analysis. The transcriptomic identity and genomic mutation profiles are integrated into a single-cell multiomic hub, enabling direct mapping between single-cell genotype and transcriptional phenotype (e.g., linking somatic mutation burdens and CNVs to specific cellular states).

For the transcriptomic component, raw 10x Genomics sequencing data were processed using **Seurat** to generate gene-by-cell expression matrices after alignment to the GRCh38 reference genome[2]. Cells were subjected to standard quality-control filtering based on sequencing depth, number of detected genes, and the proportion of mitochondrial transcripts. The filtered expression matrix was normalized, and highly variable genes were selected for downstream dimensionality reduction and clustering. Principal component analysis was performed, followed by construction of a nearest-neighbor graph and UMAP visualization. Cell-type identities were assigned by comparing ResolveOME transcriptomic profiles with a reference single-cell RNA-seq dataset containing major brain cell populations. This reference-based mapping enabled identification of microglia and other cell types and provided the transcriptomic labels used for downstream integration with genomic data.

For the genomic component, sequencing reads generated from PTA-amplified single-cell DNA were aligned to the GRCh38 reference genome and subjected to standard quality-control procedures to assess sequencing depth, genome coverage, amplification uniformity, and other library-quality metrics. Somatic single-nucleotide variants and small insertions/deletions were identified using **SCAN2**, with filtering strategies designed to account for technical artifacts introduced during single-cell whole-genome amplification[3]. High-confidence somatic variants were subsequently used to estimate mutation burden after correction for callable genomic regions and detection sensitivity. Single-base substitutions were summarized in the SBS96 trinucleotide context, whereas small insertions and deletions were classified using standard indel mutation categories. Mutational spectra were further decomposed into known COSMIC mutational signatures to characterize the mutational processes operating in individual cells. Shared somatic mutations across cells were additionally used to examine clonal relationships and infer lineage structure. Finally, transcriptomic cell identities were integrated with the corresponding PTA-derived genomic profiles, enabling somatic mutation burden, mutational signatures, and clonal architecture to be evaluated in a cell-type-specific manner.

Cell-type annotation and somatic mutation profiling using ResolveOME

To evaluate whether transcriptomic information generated by ResolveOME could be used to identify individual brain cell types, we first projected the ResolveOME transcriptomic profiles onto a reference single-cell RNA-seq dataset containing major brain cell populations (**Figure 3A-B**). ResolveOME-derived cells mapped to several distinct reference populations, including astrocytes, oligodendrocytes, neurons, and microglia. A subset of cells showed clear correspondence with the reference microglial population and was therefore selected for subsequent single-cell genomic analysis.

We next characterized the somatic mutation profiles of four transcriptomically identified microglial cells using their matched PTA whole-genome sequencing data. The SBS96 spectra showed broadly similar mutational patterns across cells, with **C>T substitutions representing a prominent component of the mutation spectrum**, although the overall mutation burden and relative contributions of individual substitution classes varied among cells (**Figure 3C**). COSMIC signature decomposition further indicated that the SBS profiles were largely explained by a shared set of mutational signatures, with **SBS5 contributing substantially across all four cells**, together with variable contributions from additional signatures (**Figure 3D**). The total number of SBS mutations also differed among individual microglial cells, indicating cell-to-cell heterogeneity in somatic mutation burden.

Analysis of small insertions and deletions revealed additional heterogeneity among the same cells (**Figure 3E**). Most indels occurred within repetitive sequence contexts, with prominent contributions from short insertions and deletions associated with homopolymer tracts. The absolute indel burden varied considerably across cells, with one microglial cell showing a markedly higher number of indels than the others (**Figure 3F**). These observations indicate that indel profiling with the current ResolveOME workflow may require additional optimization, particularly to distinguish true biological variation from potential technical artifacts.

Together, these results demonstrate that **ResolveOME transcriptomic profiles can be used to assign cell identity while matched PTA-derived genomic data provide sufficient information for single-cell somatic mutation and mutational-signature analysis**. This integrated strategy therefore enables genomic alterations to be directly linked to transcriptionally defined cell populations within the same tissue.

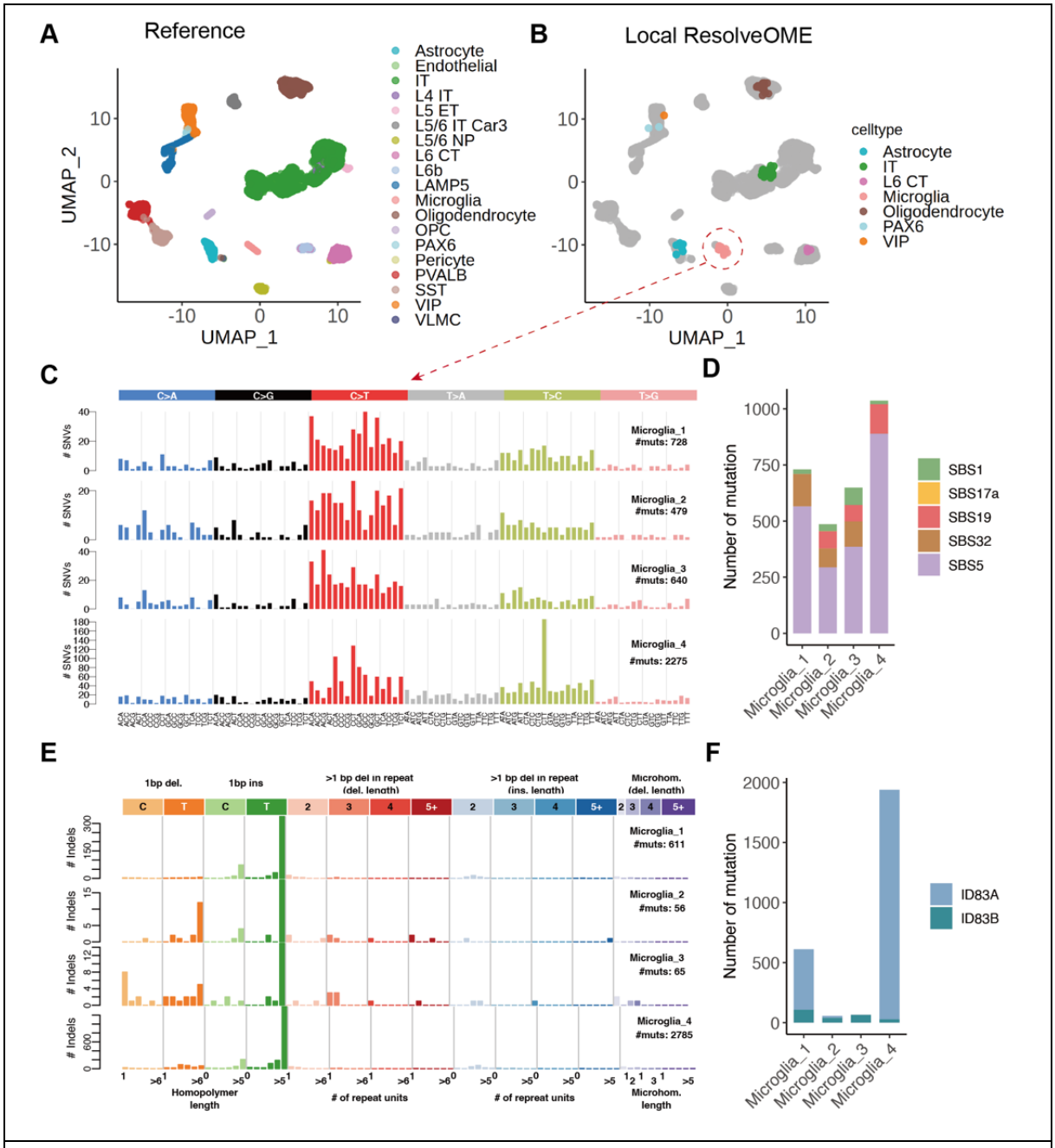


Figure 3. Cell-type annotation and mutational profiles of microglia identified by local ResolveOME analysis. (A) UMAP visualization of the reference single-cell transcriptomic dataset, with cells colored according to annotated brain cell types. **(B)** Projection of cells generated by the local ResolveOME workflow onto the reference UMAP. Assigned cell identities are indicated by color, whereas reference/background cells are shown in gray. The dashed circle highlights microglial cells selected for downstream genomic analysis. **(C)** Single-base substitution (SBS) mutational spectra of four selected microglial cells. Mutations are displayed in the 96-trinucleotide-context format and grouped into the six substitution classes: C>A, C>G, C>T, T>A, T>C, and T>G. **(D)** Decomposition of the SBS spectra into COSMIC mutational signatures. Stacked bars indicate the estimated number of mutations attributed to each SBS signature in individual cells. **(E)** Small insertion and deletion (indel) spectra of the same microglial cells using the COSMIC ID83 classification. Indels are grouped into 1-bp deletions, 1-bp insertions, >1-bp deletions at repetitive sequences, >1-bp insertions at repetitive sequences, and microhomology-mediated deletions. **(F)** Contributions of indel mutational signatures to the total indel burden in each cell, with stacked bars representing the estimated number of mutations assigned to individual ID signatures.

References

1 Marks, J. *et al.* Unifying comprehensive genomics and transcriptomics in individual cells to illuminate oncogenic and drug resistance mechanisms. *bioRxiv* (2023).

2 Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol* **36**, 411–420 (2018).

3 Luquette, L. J. *et al.* Single-cell genome sequencing of human neurons identifies somatic point mutation and indel enrichment in regulatory elements. *Nat Genet* **54**, 1564–1571 (2022).