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Single-cell RNA sequencing decodes cell-type-specific pathogenesis in Alzheimer's disease

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Abstract:

Alzheimer's disease (AD) is the most prevalent neurodegenerative dementia, characterized by progressive cognitive decline, amyloid-beta (Aβ) plaques, and neurofibrillary tangles. Its cell-type-specific pathogenic mechanisms remain unclear due to conventional bulk sequencing's limitation of averaging gene expression across heterogeneous brain tissues, hindering curative therapy development. Single-cell RNA sequencing (scRNA-seq) has emerged as a transformative tool to dissect AD's cellular heterogeneity, enabling high-resolution analysis of cell-type-specific transcriptional changes. This paper synthesizes key scRNA-seq findings in AD: it reveals region-dependent vulnerability of excitatory and inhibitory neurons, leading to disrupted excitatory-inhibitory (E/I) balance, identifies dynamic transitions of disease-associated microglia (DAM) from protective phagocytosis to maladaptive neuroinflammation, and uncovers disease-associated astrocytes (DAA) contributing to pathology via excitotoxicity, inflammation, and lipid dysregulation. It also discusses scRNA-seq limitations (e.g., postmortem sample reliance, high costs) and proposes future directions like spatial transcriptomics integration. By summarizing scRNA-seq-driven insights, this paper provides a foundation for cell-type-specific therapeutic strategies and precision medicine in AD.

Keywords: Alzheimer's disease; single-cell RNA sequencing; cell-type-specific pathogenesis; disease-associated microglia.

1. Introduction

Alzheimer's disease (AD) poses an enormous global health burden, affecting over 50 million people worldwide and projected to triple by 2050. Clinically, it manifests as progressive decline in memory, cognitive function, and executive abilities, ultimately leading to loss of independence Pathologically, AD

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is defined by extracellular Aβ plaques and intracellular tau tangles, but conventional bulk sequencing techniques—which average gene expression across heterogeneous brain tissues—have failed to resolve the cell-type-specific mechanisms driving these pathologies This limitation has hindered the development of curative therapies, as most anti-amyloid drugs have shown limited clinical efficacy, highlighting the need to dissect AD's complexity at the single-cell level

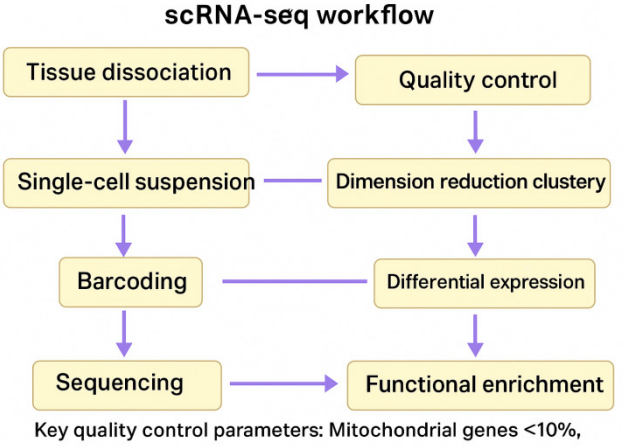
Internationally, scRNA-seq has revolutionized AD research by uncovering previously unrecognized cellular heterogeneity. Thanks to the integration of microfluidics and high-throughput sequencing, the latest research can now simultaneously capture millions of cells within a single individual sample, providing unprecedented resolution for AD studies.Single-cell level analysis was conducted on 1.3 million cells from six brain regions of Alzheimer's disease (AD) and control samples, and it was found that there was a region-dependent loss of excitatory and inhibitory neuronal subtypes. Two independent studies have discovered for the first time disease-related microglia (DAM), a subtype of microglia that is enriched around beta amyloid (Aβ) plaques and whose activation process depends on the TREM2 pathway. In the field of astrocyte research, early studies proposed a binary model of A1/A2 astrocytes, and subsequent single-cell RNA sequencing (scRNA seq) studies further refined it into a broader state spectrum, including disease-related astrocytes (DAA) associated with the pathological process of AD. In the scRNA seq study targeting AD, the transcriptome changes of neurons and glial cells in AD patients were analyzed to verify the phenomenon of excitatory inhibitory (E/I) imbalance and DAM dysfunction, providing data support for the pathological mechanism research at the cellular level of AD. scRNA-seq data is later integrated from Chinese AD cohorts, revealing population-specific cellular signatures. Despite these advances, gaps remain—such as unclear spatial-temporal dynamics of cell-type-specific changes and lack of in vivo validation of in vitro findings.

The core motivation of this review is to address the gap between fragmented scRNA-seq findings and a unified understanding of AD's cellular pathogenesis. Conventional bulk sequencing obscures cell-type-specific responses. Early scRNA-seq studies focused on individual cell types, such as neurons or microglia, in isolation. This review aims to synthesize these discrete insights into a comprehensive framework. By integrating these components, this review provides a holistic view of how scRNA-seq is reshaping AD research and guiding therapeutic development[1-3].

2. Single-Cell Sequencing Applications and Cell-Type-Specific AD Pathogenesis

2.1 Introduction of Single-Cell Sequencing Technology and Applications in Neuroscience

Single-cell RNA sequencing (scRNA-seq) profiles the transcriptome at individual cell levels on a large scale beyond the limitations of bulk sequencing. scRNA-seq has the following workflow. Isolation and barcoding. Single cells are isolated from tissue sections using microfluidic or droplet-based platforms. Each single cell is tagged with a unique molecular barcode during reverse transcription. The barcode allows sequencing data to be traced back to its originating cell. Library preparation and sequencing. Barcoded cDNA is amplified to a sufficient amount. After amplification, the transcriptome of single cells is generated via high-throughput sequencing. This material is tiny, hence the name single-cell RNA sequencing. Bioinformatic analysis. Raw data undergo quality control and alignment to a reference genome. Cell-specific expression profiles are generated via barcodes. The subsequent steps include dimensionality reduction, clustering based on transcriptional similarity, and cell-type annotation based on marker genes. Data interpretation. Transcriptional patterns of interest are related to cellular functions or disease states. Novel cell subtypes or states can be discovered.



The biggest advantage of scRNA-seq is its ability to resolve cellular heterogeneity. It is well known that gene expression in bulk sequencing is an averaged signal from all cells in a tissue. scRNA-seq converts this averaged signal into a high-resolution cellular atlas. This high resolution makes scRNA-seq able to detect subtle but critical changes in each cell type. This is a big limitation of bulk sequencing in the AD brain. The AD brain consists of various neurons, microglia, astrocytes, and other cell types.

scRNA-seq converts this averaged signal into a high-resolution cellular atlas. It allows to observe cell-type-specific transcriptional changes directly and discover novel disease-associated cell states that are not distinguishable by bulk sequencing.

In recent years, scRNA-seq has become an essential tool in neuroscience. It has revealed cellular diversity in the healthy brain. For example, more than 100 neuronal subtypes in the mouse cortex have been discovered using scRNA-seq. In AD research, scRNA-seq has been widely used. For example, in a multi-region study, 1.3 million cells from AD and control brains were profiled. Region-dependent neuronal vulnerability was discovered. In another line of research, large-scale integration of single-cell datasets was performed. Various specific astrocyte subtypes in the central nervous system were discovered and linked to different central nervous system disorders, including AD [4,5]. These works laid the foundation for interpreting cell-type-specific changes in AD neuropathology.

2.2 Cell-Type-Specific Pathogenic Insights from scRNA-seq in AD

2.2.1 Neuronal changes: disrupted E/I balance and regional vulnerability

Neural circuits are responsible for brain computation. Dysfunction of neural circuits drives cognitive decline in AD. Neural circuits are composed of excitatory and inhibitory neurons. Excitatory neurons send signals between neurons. Inhibitory neurons dampen neural activity. Neural circuits are region-specific. Different regions have different computational tasks. For example, in the cortex, computation relies on the dynamic balance between excitatory pyramidal neurons and inhibitory interneurons. Excitatory pyramidal neurons drive signal transmission. Inhibitory interneurons provide circuit control by dampening neural activity. AD disrupts this balance in a cell-type- and region-specific manner.

Two major features of neuronal pathology in AD have been revealed by ScRNA-seq studies. Spatial-temporal progression. ScRNA-seq reveals that excitatory neuronal subtypes are differentially vulnerable across brain regions. Excitatory neuron clusters are depleted in the entorhinal cortex and hippocampus, which are major memory-related regions. Some inhibitory interneurons also exhibit region-restricted loss, such as somatostatin-positive interneurons in the prefrontal cortex. AD's E/I imbalance thus appears to be region-dependent, not spatially global. This supports the notion that neuronal pathology in AD is spatially heterogeneous.

Molecular mechanisms underlying this region-dependent E/I imbalance. ScRNA-seq has revealed that inhibitory interneurons downregulate genes involved in their stabilization, such as GABA synthesis and transport genes. This weakens the function of inhibitory circuits. Excitatory pyramidal neuron clusters in the entorhinal cortex and hippocampus show tau-related synaptic dysfunction signatures. Specifically, they downregulate genes expressed in synaptic vesicles, which impair long-range excitatory connectivity. ScRNA-seq also identified astrocyte-derived glutamate transporter (EAAT2) downregulation in neurons via integrated neuronal-glial scRNA-seq. This impairs the clearance of the excitatory neurotransmitter and increases excitotoxic stress on neurons. Reactive glia then release cytokines and complement component C3, accelerating synaptic pruning and further destabilizing circuits. Altogether, these cellular mechanisms linking vulnerability at the circuit level have been revealed by scRNA-seq studies on AD [6-9].

2.2.2 Microglial changes: dynamic states of disease-associated microglia

Microglia are the resident immune cells in the brain. ScRNA-seq revealed that DAM, a subset of disease-associated microglia critical to pathogenesis, is dynamically enriched around β-amyloid plaques and possesses a transcriptional program distinct from homeostatic microglia. Homeostatic microglia express P2ry12, Tmem119, and Cx3cr1. DAM upregulates Apoe, Trem2, Cd9, Tyrobp, and Lpl, which are involved in phagocytosis and lipid metabolism. This reprogramming is undetectable by bulk analyses. ScRNA-seq thus revealed a unique property of DAM [10].

ScRNA-seq also shows that DAM undergoes multi-stage maturation. The process can be simplified into two steps. The first step is partially TREM2 independent. It involves upregulation of lipid-sensing and early plaque recognition genes. The second step is strongly TREM2 dependent. It allows DAM to gain a fully activated phenotype with enhanced phagocytic and lysosomal functions[7]. In early AD, DAM plays a protective role by containing Aβ accumulation. With disease progression, DAMs become chronically activated. They downregulate homeostatic markers and persist in a chronically inflammatory state with expression of Cst7, Clec7a and Itgax. This switches their function from beneficial clearance to a maladaptive neuroinflammation[11].

Mechanistically, DAMs are linked to key AD pathogenic pathways. TREM2-APOE axis regulates DAM differentiation and survival. TREM2 signaling modulates lipid metabolism, cytoskeletal rearrangements and inflammatory cascades. APOE is a major AD risk gene and is required for the differentiation of mature DAM with phagocytic

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function[7]. Besides, DAMs are involved in complement activation and induce excessive synaptic pruning. This destabilizes E/I circuits. This is consistent with recent reports that synaptic loss may be an early driver of cognitive decline. However, DAM is a "double-edged sword". DAM's chronic activation will secrete pro-inflammatory cytokines and reactive oxygen species and amplify neurotoxicity. DAM's interactions with astrocytes and neurons form pathological feedback loops and amplify dysfunction [10,11].

2.2.3 Astrocytic changes: heterogeneity and disease-associated subtypes

Astrocytes used to be considered as passive support cells. ScRNA-seq has revealed their high heterogeneity and active role in AD pathogenesis. Early studies suggested a simple A1/A2 astrocyte dichotomy. A1 astrocytes are neurotoxic and induced by pro-inflammatory microglia. A2 astrocytes are neuroprotective and associated with repair. However, scRNA-seq in human and mouse AD brains revealed a much more diverse spectrum of astrocytic states, including subsets enriched for complement components such as C3 and Serping1, as well as subsets enriched for trophic support genes. This shows that the A1/A2 model is overly simplified. In human AD brains, most reactive astrocytes displayed an A1-like pro-inflammatory state. A2-like supportive states were extremely rare. This astrocytic imbalance aggravated synaptic loss [3,12].

ScRNA-seq also discovered DAAs, a novel subset of astrocytes localized near Aβ plaques. DAAs upregulate Apoe, Clu and Gfap. They differentiate through the gradual reprogramming of homeostatic astrocytes in a similar manner to DAM's transformation. Longitudinal scRNA-seq in transgenic AD mice demonstrated an increase in abundance of DAA with AD progression. Their spatial clustering around plaques coincides with DAM's enrichment, suggesting that microglia and astrocytes are functionally dependent on each other in the AD brain. A local inflammatory niche orchestrated through crosstalk via cytokines, complement proteins, and lipid mediators amplifies inflammation and drives neurodegeneration[12].

Mechanistically, astrocytes contribute to AD pathogenesis through at least four major pathways (Figure 2.3). One is excitotoxicity. Astrocytes regulate glutamate homeostasis by uptaking glutamate from neurons into astrocytes through glutamate transporters, such as EAAT2 and EAAT1. Downregulation of these genes leads to a decrease in glutamate transport, which in turn causes neuronal stress. The second is inflammation. Reactive astrocytes produce pro-inflammatory cytokines such as IL-1β and TNF-α, as well as C3, which tags synapses for elimination by microglia. The third is lipid dysregulation. Astrocytes are the major source of brain APOE. In AD, astrocytes show altered expression of genes involved in cholesterol transport and lipid handling, which in turn affect Aβ aggregation and clearance. The fourth is vascular dysfunction. Downregulation of genes encoding vascular support functions in reactive astrocytes may promote BBB leakage and reduced perfusion. Thus, astrocytes are active modulators of AD pathogenesis rather than simple bystanders[3,12].

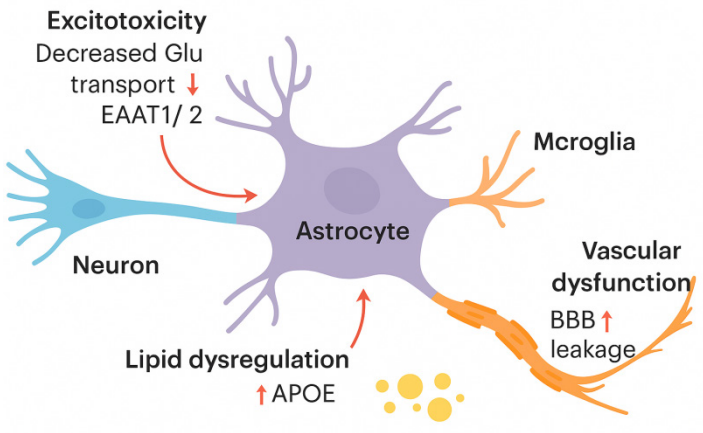

Figure2.3

2.2.4 Changes in oligodendrocytes: myelination impairment and disease-associated states

Oligodendrocytes are responsible for myelinating axons in the central nervous system. In recent years, scRNA-seq has uncovered that oligodendrocytes also exhibit transcriptional changes in AD, which affect myelination integrity and neuronal signal conduction in addition to the more well-studied effects in neurons and glia, such as

microglia. ScRNA-seq analysis revealed that oligodendrocyte precursor cells (OPCs) in AD brains exhibit reduced proliferation and differentiation potential. Compared to control samples, oligodendrocytes from AD brains show downregulation of multiple genes involved in cell cycle progression and oligodendrocyte maturation (PDGFRA, SOX10) from OPCs from AD brains, leading to a decrease in mature oligodendrocytes, which are responsible for forming myelin sheaths around axons. Mature oligodendrocytes in AD also show altered expression of multiple myelin-related genes (MBP, PLP1), which are responsible for synthesizing and repairing myelin. The downregulation of these genes leads to myelin loss in several key brain regions (corpus callosum, hippocampus), which are associated with cognitive function.

In addition, scRNA-seq also discovered subsets of disease-associated oligodendrocytes (DAO) in AD brains. DAO cells upregulate multiple genes involved in oxidative stress response and inflammation, such as HMOX1 and IL6R. Their activation also further impairs oligodendrocyte function and promotes myelin degradation. Meanwhile, myelin loss further impairs neuronal axon integrity and signal transmission, leading to cognitive decline. Besides, DAO also shows multi-cell crosstalk in the AD brain: DAO secretes inflammatory factors to activate microglia, and they also receive signals from reactive astrocytes to further inhibit their maturation. All these interactions form a pathological loop to further impair DAO maturation and AD pathogenesis. These scRNA-seq-derived findings revealed that oligodendrocytes are an underestimated player involved in AD pathogenesis. In addition to neurons, microglia, and astrocytes, scRNA-seq further extended our understanding of cellular complexity involved in AD pathogenesis and provided a more holistic view of how multiple cell types impair neuronal function in AD[13].

2.3 Limitations of Current scRNA-seq Application in AD Research.

Although revolutionary, scRNA-seq also has the following limitations in AD research. Firstly, most scRNA-seq datasets were derived from postmortem brains. Postmortem samples are limited in cohort size, which further limits the statistical power of analysis. Meanwhile, postmortem samples may suffer from postmortem degradation, which may further modify transcriptional profiles. In vitro iPSC-derived neurons are often less complex than in vivo neurons. Therefore, these models may fail to recapitulate in vivo brain conditions, which limits the translational significance of results. The collection of scRNA-seq datasets is technically challenging and costly. Platforms like Dropseq require a specific droplet sequencer. Bioinformatic analysis requires high computing resources. Therefore, it limits the possibility of conducting large-scale or longitudinal studies. Different scRNA-seq platforms like 10x Genomics or Smart-seq2 may generate different data formats. Therefore, it limits the comparison between different studies. Finally, scRNA-seq only reflects transcriptional changes. It may further limit the exploration of post-transcriptional modification or protein-protein interaction, which are critical to explore AD's functional pathology.

3. Conclusion

Single-cell RNA sequencing has resolved cell-type-specific heterogeneity and dynamic changes underlying Alzheimer's disease pathogenesis. In this review, we summarize key insights derived from scRNA-seq studies: it clarifies that excitatory and inhibitory neurons are region-dependently vulnerable, leading to E/I balance disruption; identifies disease-associated microglia (DAM) transition from phagocytic protection to maladaptive neuroinflammation; and identifies disease-associated astrocytes (DAA) impair neuronal function via excitotoxicity, inflammation and lipid dysregulation. It also identifies limitations of current scRNA-seq approaches, like postmortem samples limitation, technical limitation and data integration limitation. Because these results were obtained using scRNA-seq of bulk sequencing data averaged across cells and AD data generated from cellularly diverse populations, our work provides a bridge to link both AD and its cell-type-specific therapies (e.g., modulating the inflammatory state of DAM or rescuing astrocyte glutamate transport, etc.). In the future, scRNA-seq combined with spatial transcriptomics will allow studying cellular states in their native tissue environment, and epigenomic profiling will provide regulatory mechanisms underlying transcriptional changes. It will also be a priority to identify early cellular biomarkers for preclinical AD and to validate in vitro findings in vivo. By building on the current work and addressing some of the limitations, scRNA-seq will guide AD research toward faster translation of mechanistic findings into precision medicine strategies for this devastating disease.

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