Acta Pharmaceutica Sinica B 2025;15(4):2290–2292

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

HIGHLIGHT

Multi region dissection of Alzheimer's brain at single cell level

KEY WORDS

Alzheimer's disease;
snRNA-seq;
Neuron;
Astrocytes;
Cellular diversity;
Cognitive resilience

Alzheimer's disease (AD) is the leading cause of dementia worldwide. Traditionally, pathological studies have concentrated on the abnormal buildup of amyloid β (Aβ) plaques and neurofibrillary tangles (NFTs), which arise from the excessive phosphorylation of tau proteins in the brain[1,2]. Despite extensive research, these efforts have not yet identified an effective molecular target for AD treatment. A significant challenge is that existing studies have not fully captured the complex cellular microenvironment that contributes to Aβ and tau protein deposition. A recent study, published in Nature, bridges this gap by examining six distinct brain regions from 283 post-mortem human brain samples derived from 48 individuals, both with and without AD. Utilizing single-cell mRNA sequencing (scRNA-seq), the study establishes a comprehensive dataset comprising 1.3 million cells[3]. This innovative approach provides novel insights into the cellular diversity and dynamic interaction underlying AD pathology.

scRNA-seq is a powerful technique for unraveling cellular diversity. It allows for the identification and tracking of various brain cell types, including excitatory neurons, inhibitory neurons, and glial cells, among others. Although several scRNA-seq studies have been published on examination of human or mouse brain samples suffering AD, these studies have yet to deliver a comprehensive cellular diversity landscape spanning multiple brain regions within a single investigation[4,5]. Currently, due to the unclear pathogenesis despite numerous hypotheses, the absence of validated targets, and the lack of effective therapies, a cure for AD remains elusive, and current treatments are mostly symptomatic[6,7]. Although some brain regions have been investigated in AD patients, these studies have typically focused on single regions or involved only a few individuals[8,9]. By conducting an in-depth cell type-specific analysis of brain samples from healthy elderly individuals and AD patients, the new study aims to construct a comprehensive single-cell transcriptional map of the aging brain[3]. The goal is to reveal cellular heterogeneity in the pathological process of AD, explore specific changes in cell types across different brain regions, and understand how these changes are associated with cognitive impairment.

To elucidate the cellular diversity across multiple regions implicated in the progression of AD, Kellis et al. examined six brain regions within 283 post-mortem brain samples[3]. These regions included the entorhinal cortex (EC), hippocampus (HC), anterior thalamus (TH), angular gyrus (AG), midtemporal cortex (MT), and prefrontal cortex (PFC)[3]. The study involved 48 individuals, among whom 26 had been diagnosed with AD. A total of 76 high-resolution subtypes were defined across 14 major cell type groups, including 32 subtypes of excitatory neurons and 23 subtypes of inhibitory neurons. The compositional differences of major cell types in the six brain regions in this comprehensive study have established a multi-regional AD cell atlas, which not only enhances our understanding of the cellular structure of the human brain but also provides new insights into the early diagnosis and therapeutic intervention of AD.

The regional diversity of excitatory and inhibitory neurons was found in the human brain[3]. Excitatory neuron subtypes (12 subtypes) were found to be either highly region-specific to the hippocampus (HC), entorhinal cortex (EC), and anterior thalamus (TH) or predominantly shared across neocortical regions. Meanwhile, the majority of inhibitory neuron subtypes (22 out of 23 subtypes) were observed across all five cortical regions. Notably, the TH contained a unique, thalamus-specific inhibitory subtype

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.12.010

characterized by genes involved in neurite outgrowth. It was intriguing to find differences in cellular communication between the thalamus-specific excitatory subtype and the neocortex-specific inhibitory subtype, suggesting a distinctive role for the thalamus in neuronal communication. Further analysis revealed that subtypes of neurons in specific regions, such as hippocampal CA1 pyramidal neurons and entorhinal cortex-specific subtypes (L2 RELN lateral EC, L3 RELN, L5, and L2/3 TOX3TTC6 neurons), were reduced in individuals with AD. These vulnerable excitatory neurons share gene expression profiles related to the Reelin signaling pathway, which were validated in both human and mouse models of AD. These findings provide evidence of regional diversity of neurons in AD with specific neuronal subtypes.

Similarly, diversity was identified in glial cells across brain regions, with astrocytes showing the highest degree of regional heterogeneity[3]. Among all glial cells, astrocytes were found to have subtypes that were either highly enriched in the neocortex or specifically in the thalamus. By calculating region-specific differentially expressed genes (DEGs) of major cell types in the brain regions of patients with pathological AD, this study identified that astrocytes, inhibitory neurons, and excitatory neurons had the highest number of DEGs across all regions, with the most significant changes observed in the entorhinal cortex (EC). Furthermore, AD-related genes identified by Genome-Wide Association Studies (GWAS) were found to be most highly expressed in microglia, with many showing region-specific expression patterns. These findings underscore that cellular and region-specific pathological changes in AD encompass a multitude of biological processes, highlighting the complexity of the disease's impact on the brain's cellular landscape.

The study identified differentially expressed genes (DEGs) for region-specific measurements of neurofibrillary tangle (NFT) and amyloid-β plaque burden[3]. The DEGs associated with AD pathology demonstrated the highest overlap in all cell types of the entorhinal cortex (EC) and hippocampus (HC), with the lowest overlap observed in the prefrontal cortex (PFC) and angular gyrus (AG). Notably, plaque-associated DEGs in excitatory neurons were strongly enriched for components of the aerobic transport chain, and astrocytes contained a higher number of plaque-associated DEGs, which were enriched in metallostasis. In addition, among all cell types, astrocytes were the only type that contained a high number of genes associated with cognitive resilience, including GPX3 (glutathione peroxidase 3), HMGN2 (high mobility group nucleosomal binding domain 2), NQO1 (NAD(P)H quinone dehydrogenase 1), and ODC1 (ornithine decarboxylase 1). These findings provide valuable insights into the cellular and molecular underpinnings of cognitive resilience in the context of AD, which highlight the potential role of astrocytes in modulating the brain's response to pathological changes associated with AD.

In summary, by constructing a transcriptomic atlas of six brain regions in 48 individuals with and without AD, this study identified 76 distinct subtypes of brain cells[3]. These include region-specific subtypes of astrocytes and excitatory neurons, as well as an inhibitory interneuron subpopulation unique to the thalamus and distinct from the canonical inhibitory subpopulation. Vulnerable subpopulations of excitatory and inhibitory neurons were found, and the Reelin signaling pathway was found to modulate their vulnerability. Moreover, a scalable method for discovering gene modules was developed to identify altered cell-type-specific and region-specific modules and to annotate transcriptomic differences associated with diverse pathological variables. Additionally, an astrocyte program associated with resistance to AD pathology was identified, linking choline metabolism and polyamine biosynthesis in astrocytes to preserved cognitive function in later life. However, the study acknowledges some limitations. Isotropic fractionation and read depth cut-offs may bias cell recovery based on their nuclear content, and nuclear RNA may not fully capture microglial states or localized transcriptomic changes[10]. Furthermore, the pathology burden is based on per-sample averages rather than on the spatial context of each cell. Expanding the sample size and incorporating additional data sets will enhance our understanding of region-specific alterations in AD brain. Importantly, spatial data is needed to provide location information of the subpopulations of brain cells in pathology-associated changes, providing a more constructive view of the disease's impact across different brain regions.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Grant No. 32271220).

Author contributions

Meng Mao and Chengming Wang drafted the manuscript. Xiwen Ma and Jianping Ye provided the idea and revised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Lowe VJ, Lundt ES, Albertson SM, Min HK, Fang P, Przybelski SA, et al. Tau-positron emission tomography correlates with neuropathology findings. *Alzheimers Dement* 2020;**16**:561−71.
2. Gallego-Rudolf J, Wiesman AI, Pichet Binette A, Villeneuve S, Baillet S, PREVENT-AD Research Group. Synergistic association of Aβ and tau pathology with cortical neurophysiology and cognitive decline in asymptomatic older adults. *Nat Neurosci* 2024;**27**:2130−7.
3. Mathys H, Boix CA, Akay LA, Xia Z, Davila-Velderrain J, Ng AP, et al. Single-cell multiregion dissection of Alzheimer's disease. *Nature* 2024;**632**:858−68.
4. Gerrits E, Brouwer N, Kooistra SM, Woodbury ME, Vermeiren Y, Lambourne M, et al. Distinct amyloid-β and tau-associated microglia profiles in Alzheimer's disease. *Acta Neuropathol* 2021;**141**:681−96.
5. Morabito S, Miyoshi E, Michael N, Shahin S, Martini AC, Head E, et al. Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease. *Nat Genet* 2021;**53**:1143−55.
6. Du X, Wang X, Geng M. Alzheimer's disease hypothesis and related therapies. *Transl Neurodegener* 2018;**7**:2.
7. Barrera-Ocampo A. Monoclonal antibodies and aptamers: the future therapeutics for Alzheimer's disease. *Acta Pharm Sin B* 2024;**14**:2795−814.
8. Sziraki A, Lu Z, Lee J, Banyai G, Anderson S, Abdulraouf A, et al. A global view of aging and Alzheimer's pathogenesis-associated cell population dynamics and molecular signatures in human and mouse brains. *Nat Genet* 2023;**55**:2104−16.
9. Gabitto MI, Travaglini KJ, Rachleff VM, Kaplan ES, Long B, Ariza J, et al. Integrated multimodal cell atlas of Alzheimer's disease. *Nat Neurosci* 2024;**27**:2366−83.
10. Thrupp N, Sala Frigerio C, Wolfs L, Skene NG, Fattorelli N, Poovathingal S, et al. Single-nucleus RNA-Seq is not suitable for detection of microglial activation genes in humans. *Cell Rep* 2020;**32**:108189.

Meng Mao[a], Chengming Wang[a], Xiwen Ma[a], Jianping Ye[a,b,*]
[a]*Institute of Trauma and Metabolism, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou 450007, China*
[b]*Tianjian Laboratory of Advanced Biomedical Sciences, Academy of Medical Sciences, Zhengzhou University, Zhengzhou 450001, China*

*Corresponding authors.
E-mail addresses: yejianping@zzu.edu.cn (Jianping Ye)

Received 1 November 2024
Received in revised form 9 December 2024
Accepted 11 December 2024