Report Title

Introduction

Introduction:

Alzheimer's Disease (AD) is a multifaceted neurodegenerative disorder characterized by intricate cellular and molecular heterogeneity within the brain. Understanding the diverse cell populations and their implications in AD pathophysiology has been significantly enhanced by recent advancements in single-cell multi-omic analysis. This research delves into the exploration of single-cell heterogeneity in Alzheimer's disease through the lens of a comprehensive multi-omic approach.

The objective of this study is to investigate the perturbations in multicellular communities within the aging human brain and Alzheimer's disease by analyzing differentially expressed genes, particularly focusing on oligodendrocytes. By identifying shared enriched pathways among cell subsets and comparing cell clusters with existing datasets, the research endeavors to employ a regression-based consensus model (CelMod) to extend the findings to bulk data. This innovative approach is pivotal in elucidating the unique characteristics of individual cell types and employing topic models to capture expression programs.

The significance of this research lies in its potential to provide valuable insights into the heterogeneity of Alzheimer's disease at the single-cell level, thereby contributing to a more profound comprehension of the disease pathogenesis. By shedding light on cellular communities and their associations with AD traits, the study aims to pave the way for novel therapeutic strategies and precision medicine interventions targeting specific cell subsets to mitigate cognitive decline and enhance patient outcomes.

Through an integrative analysis of multi-omic data from postmortem brain tissue and the utilization of mediation analysis, this research intends to reveal the temporal links connecting cellular communities, pathology development, and cognitive symptoms in Alzheimer's disease. By adopting a network approach, the study not only generates actionable hypotheses for future investigations but also underscores the critical importance of grasping the underlying cellular networks in AD and other neurodegenerative disorders.

In essence, this research advances the current understanding of AD pathophysiology by providing a holistic view of the complex cellular interactions dictating disease progression. By delineating the intricate single-cell heterogeneity in Alzheimer's disease, this study not only unveils novel insights but also sets the stage for the development of precise therapeutic interventions targeting specific cell subtypes, thereby offering a promising avenue for enhancing patient care in the realm of

neurodegenerative diseases like Alzheimer's.

Literature

pathology and cognitive decline. The study employs a multi-omic approach and mediation analysis to understand the temporal links between cellular communities, pathology appearance, and cognitive symptoms. Utilizing postmortem brain tissue profiling, the researchers propose a unified view of the brain's cellular ecosystems, emphasizing the importance of understanding cellular communities in the pathogenesis of AD and similar disorders.

Data for the research were derived from two clinical-pathologic cohort studies, the Religious Orders Study (ROS) and the Memory and Aging Project (MAP), both of which underwent deep ante- and postmortem characterization. The longitudinal cognitive assessments and clinical diagnosis of AD at the time of death were carried out by experts in dementia, highlighting the rigor and precision of the data collection process.

In addition to highlighting the experimental methods, the literature review refers to various methodologies and databases employed in the study, such as UMAP for data visualization, DoubletFinder for doublet detection, and the Molecular Signatures Database (MSigDB) hallmark gene set collection. Furthermore, the study aligns with consensus recommendations for the postmortem diagnosis of Alzheimer's disease, emphasizing the need for standardized approaches in studying neurodegenerative disorders.

The review also integrates references to previous research on AD, citing studies on the amyloid hypothesis, amyloid and tau association with cognition, and their impact on prospective cognitive decline in older individuals. These studies have contributed significantly to the understanding of AD pathophysiology and its implications for cognitive function, providing a solid foundation for the current research.

Overall, the literature review provides a comprehensive overview of the existing research related to single-cell heterogeneity in AD, emphasizing the significance of a multi-omic approach and mediation analysis in uncovering new insights into the cellular communities involved in different aspects of the disease. The study's emphasis on a unified view of cellular ecosystems in the brain highlights the potential for advancements in elucidating the complex mechanisms underlying AD.

Discussion

ive symptoms, indicating that changes in specific cell subsets partially mediate the known link between tau pathology and cognitive decline.

Strengths:

1. **Novel Approach**: The use of a multi-omic approach highlights the innovative nature of the research, providing a comprehensive understanding of the cellular heterogeneity in AD.
2. **Significance of Unified View**: Emphasizing the importance of a unified view of cellular ecosystems offers valuable insights into potential therapeutic targets and diagnostic markers for AD.
3. **Proposed Testable Hypotheses**: The study's use of mediation analysis has proposed testable hypotheses for future research, indicating the potential for further investigations into the role of specific cellular communities in AD pathology.

Weaknesses:

1. **Indirect Measurement of Temporal Links**: The study acknowledged the limitation of not directly measuring the temporal links between cellular communities, pathology, and cognitive symptoms, potentially limiting the understanding of dynamic changes over time.
2. **Reliance on Postmortem Brain Tissue**: Utilizing postmortem brain tissue may not fully capture the dynamic nature of cellular ecosystems in the progression of AD, potentially leading to biased results.
3. **Need for Further Validation**: While the proposed hypotheses are insightful, further validation through experimental and longitudinal studies is necessary to confirm the relationships between cellular subsets and AD pathology.

Future Directions:

1. **Longitudinal Studies**: Conducting longitudinal studies to directly measure the temporal links between cellular communities and cognitive decline in living subjects could provide a more comprehensive understanding of the disease progression.
2. **Dynamic Imaging Techniques**: Implementing live-cell imaging or other dynamic approaches to capture real-time changes in cellular ecosystems associated with AD could offer valuable insights into the disease trajectory.
3. **Integration of Multi-Omic Data**: Further integration of multi-omic datasets with clinical and cognitive assessments could enhance the understanding of the molecular mechanisms underlying AD heterogeneity.

In summary, the study significantly contributes to the field of AD research by highlighting the importance of a unified view of cellular ecosystems and proposing testable hypotheses for further investigation. However, the limitations in directly measuring temporal links and the reliance on postmortem brain tissue call for additional studies to validate the findings and advance our understanding of single-cell heterogeneity in Alzheimer's Disease.

Idea

Problem:
Investigate the impact of sex-specific transcriptional responses in Alzheimer's disease pathology across multiple brain cell types identified through single-cell transcriptomic analysis, and analyze the functional implications of myelination-related processes in the pathophysiology of the disease.

Rationale:

The single-cell transcriptomic analysis of Alzheimer's disease has highlighted the substantial differences in transcriptional responses between sexes in several brain cell types, particularly in oligodendrocytes. However, the functional significance of these sex-specific transcriptional responses in Alzheimer's disease pathology remains to be fully elucidated. Furthermore, the recurrent perturbation of myelination-related processes across multiple cell types suggests a potential key role of myelination in Alzheimer's disease pathophysiology. Investigating the impact of sex-specific transcriptional responses and understanding the functional implications of myelination-related processes will provide crucial insights into the sex disparities in Alzheimer's disease and potentially uncover novel therapeutic targets for intervention.

Method

Method:

1. Data Collection and Preprocessing:

- Obtain single-cell transcriptomic data from publicly available datasets or conduct new single-cell RNA sequencing (scRNA-seq) experiments on post-mortem brain tissue samples from Alzheimer's disease (AD) patients and age-matched controls, ensuring representation from both sexes.
- Preprocess the raw scRNA-seq data to remove batch effects, perform quality control, and normalize gene expression values.

2. Identification of Sex-Specific Transcriptional Responses:

- Perform differential gene expression analysis to identify genes that exhibit sex-specific transcriptional responses in different brain cell types, with a focus on oligodendrocytes.
- Utilize statistical methods to define sex-specific gene expression patterns, taking into account potential confounding factors such as age, disease severity, and post-mortem interval.

3. Functional Analysis of Sex-Specific Transcriptional Responses:

- Investigate the biological functions and pathways associated with sex-specific transcriptional responses using gene ontology enrichment analysis, pathway analysis, and network-based approaches, aiming to elucidate the molecular mechanisms underlying sex disparities in AD pathology.
- Validate the functional implications of sex-specific transcriptional responses through in vitro and in vivo experimental models, including cell culture systems and animal models, to assess their impact on myelination and other relevant processes.

4. Analysis of Myelination-Related Processes:

- Explore the perturbation of myelination-related processes across multiple brain cell types using integrated analyses of gene expression and functional annotations.
- Investigate the potential interactions between sex-specific transcriptional responses and myelination-related processes, aiming to elucidate their combined effects on AD pathophysiology.

5. Integration with Clinical and Neuropathological Data:

- Integrate the transcriptomic findings with relevant clinical and neuropathological data, including cognitive assessments, neuropathological staging, and neuroimaging data, to correlate sex-specific transcriptional responses and myelination-related processes with disease progression and clinical outcomes in AD.

Rationale:

This method integrates cutting-edge single-cell transcriptomic analysis with advanced statistical and functional approaches to comprehensively investigate the impact of sex-specific transcriptional responses and myelination-related processes in AD pathology. By leveraging publicly available datasets or conducting new experiments, the method ensures the representation of both sexes, addressing the identified sex disparities. The differential gene expression analysis, functional enrichment analysis, and experimental validation contribute to a rigorous and comprehensive understanding of the molecular mechanisms underlying sex-specific differences in AD pathophysiology. Furthermore, the integration with clinical and neuropathological data enhances the translational relevance and generalizability of the findings, laying the groundwork for identifying novel therapeutic targets and personalized treatment strategies for AD.

Experiment

Experiment:

Data Collection and Preprocessing:

- **Objective**: Obtain single-cell transcriptomic data from publicly available datasets or conduct new single-cell RNA sequencing (scRNA-seq) experiments on post-mortem brain tissue samples from Alzheimer's disease (AD) patients and age-matched controls to characterize sex-specific transcriptional responses.
- **Method**:
- Source publicly available scRNA-seq datasets from Alzheimer's disease brain samples ensuring a balanced representation of both sexes or conduct new scRNA-seq experiments on post-mortem brain tissues.
- Preprocess raw scRNA-seq data to mitigate batch effects, ensure quality control, and normalize gene expression values to establish a reliable dataset.

Identification of Sex-Specific Transcriptional Responses:

- **Objective**: Identify genes exhibiting sex-specific transcriptional responses in different brain cell types, focusing on oligodendrocytes to understand the disparities in Alzheimer's disease pathology.
- **Method**:
- Conduct differential gene expression analysis to pinpoint genes showing significant sex-specific expression patterns while considering potential confounding factors such as age and disease severity.
- Utilize statistical tools to characterize sex-specific gene expression patterns across various brain cell types comprehensively.

Functional Analysis of Sex-Specific Transcriptional Responses:

- **Objective**: Investigate the biological relevance and pathways associated with sex-specific transcriptional responses to unveil the molecular mechanisms underlying sex disparities in Alzheimer's disease pathology, with a focus on myelination-related processes.
- **Method**:
- Employ gene ontology enrichment analysis, pathway analysis, and network-based approaches to decipher the functional implications of sex-specific transcriptional responses.
- Validate the functional implications through in vitro and in vivo experimental models, evaluating their impact on myelination and other relevant processes for a holistic understanding.

Analysis of Myelination-Related Processes:

- **Objective**: Explore the perturbation of myelination-related processes across multiple brain cell types by integrating gene expression data and functional annotations to understand their contribution to Alzheimer's disease pathophysiology.
- **Method**:
- Conduct integrated analyses to elucidate the interactions between sex-specific transcriptional responses and myelination-related processes in the context of Alzheimer's disease.
- Investigate potential synergistic effects on disease progression by comprehensively analyzing myelination-related processes in different brain cell types.

Integration with Clinical and Neuropathological Data:

- **Objective**: Fuse transcriptomic findings with pertinent clinical and neuropathological data to correlate sex-specific transcriptional responses and myelination-related processes with disease progression and clinical outcomes in Alzheimer's disease.
- **Method**:
- Merge transcriptomic data with cognitive assessments, neuropathological staging, and neuroimaging data to establish robust correlations between molecular profiles and disease progression.
- Integrate clinical and neuropathological data for a comprehensive understanding of the impact of sex-specific transcriptional responses and myelination-related processes on Alzheimer's disease pathophysiology and clinical outcomes.

Rationale:

This experiment design aims to systematically investigate the impact of sex-specific transcriptional responses and myelination-related processes in Alzheimer's disease pathology. By leveraging single-cell transcriptomic analysis and advanced statistical methods, this study seeks to fill crucial knowledge gaps in understanding sex disparities in Alzheimer's disease and elucidate the functional implications of myelination processes. The integration of molecular data with clinical and neuropathological information ensures a comprehensive approach that can potentially reveal novel therapeutic targets and personalized treatment strategies for Alzheimer's disease.

More related paper

Paper 1
Title: Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease.

Abstract: The gene-regulatory landscape of the brain is highly dynamic in health and disease, coordinating a menagerie of biological processes across distinct cell types. Here, we present a multi-omic single-nucleus study of 191,890 nuclei in late-stage Alzheimer's disease (AD), accessible through our web portal, profiling chromatin accessibility and gene expression in the same biological samples and uncovering vast cellular heterogeneity. We identified cell-type-specific, disease-associated candidate cis-regulatory elements and their candidate target genes, including an oligodendrocyte-associated regulatory module containing links to APOE and CLU. We describe cis-regulatory relationships in specific cell types at a subset of AD risk loci defined by genome-wide association studies, demonstrating the utility of this multi-omic single-nucleus approach. Trajectory analysis of glial populations identified disease-relevant transcription factors, such as SREBF1, and their regulatory targets. Finally, we introduce single-nucleus consensus weighted gene coexpression analysis, a coexpression network analysis strategy robust to sparse single-cell data, and perform a systems-level analysis of the AD transcriptome.

DOI: 10.1038/s41588-021-00894-z

The impact factor: 41.307

Paper 2

Title: A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation.

Abstract: There is currently little information available about how individual cell types contribute to Alzheimer's disease. Here we applied single-nucleus RNA sequencing to entorhinal cortex samples from control and Alzheimer's disease brains (nâ■■=â■■6 per group), yielding a total of 13,214 high-quality nuclei. We detail cell-type-specific gene expression patterns, unveiling how transcriptional changes in specific cell subpopulations are associated with Alzheimer's disease. We report that the Alzheimer's disease risk gene APOE is specifically repressed in Alzheimer's disease oligodendrocyte progenitor cells and astrocyte subpopulations and upregulated in an Alzheimer's disease-specific microglial subopulation. Integrating transcription factor regulatory modules with Alzheimer's disease risk loci revealed drivers of cell-type-specific state transitions towards Alzheimer's disease. For example, transcription factor EB, a master regulator of lysosomal function, regulates multiple disease genes in a specific Alzheimer's disease astrocyte subpopulation. These results provide insights into the coordinated control of Alzheimer's disease risk genes and their cell-type-specific contribution to disease susceptibility. These results are available at http://adsn.ddnetbio.com.

DOI: 10.1038/s41593-019-0539-4

The impact factor: 28.771

Paper 3

Title: Insights into Alzheimer's disease from single-cell genomic approaches.

Abstract: Alzheimer's disease (AD) is an age-related disease pathologically defined by the deposition of amyloid plaques and neurofibrillary tangles in the brain parenchyma. Single-cell profiling has shown that Alzheimer's dementia involves the complex interplay of virtually every major brain cell type. Here, we highlight cell-type-specific molecular perturbations in AD. We discuss how genomic information from single cells expands existing paradigms of AD pathogenesis and highlight new opportunities for therapeutic interventions.

DOI: 10.1038/s41593-022-01222-2

The impact factor: 28.771

Paper 4

Title: Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease.

Abstract: The extent of microglial heterogeneity in humans remains a central yet poorly explored question in light of the development of therapies targeting this cell type. Here, we investigate the population structure of live microglia purified from human cerebral cortex samples obtained at autopsy and during neurosurgical procedures. Using single cell RNA sequencing, we find that some subsets are enriched for disease-related genes and RNA signatures. We confirm the presence of four of these microglial subpopulations histologically and illustrate the utility of our data by characterizing further microglial cluster 7, enriched for genes depleted in the cortex of individuals with Alzheimer's disease (AD). Histologically, these cluster 7 microglia are reduced in frequency in AD tissue, and we validate this observation in an independent set of single nucleus data. Thus, our live human microglia identify a range of subtypes, and we prioritize one of these as being altered in AD.

DOI: 10.1038/s41467-020-19737-2

The impact factor: 17.694

Paper 5

Title: Multicellular communities are perturbed in the aging human brain and Alzheimer's disease.

Abstract: The role of different cell types and their interactions in Alzheimer's disease (AD) is a complex and open question. Here, we pursued this question by assembling a high-resolution cellular map of the aging frontal cortex using single-nucleus RNA sequencing of 24 individuals with a range of clinicopathologic characteristics. We used this map to infer the neocortical cellular architecture of 638 individuals profiled by bulk RNA sequencing, providing the sample size necessary for identifying statistically robust associations. We uncovered diverse cell populations associated with AD, including a somatostatin inhibitory neuronal subtype and oligodendroglial states. We further identified a network of multicellular communities, each composed of coordinated subpopulations of neuronal, glial and endothelial cells, and we found that two of these communities are altered in AD. Finally, we used mediation analyses to prioritize cellular changes that might contribute to cognitive decline. Thus, our

deconstruction of the aging neocortex provides a roadmap for evaluating the cellular microenvironments underlying AD and dementia.

DOI: 10.1038/s41593-023-01356-x

The impact factor: 28.771

Paper 6

Title: Integrative in situ mapping of single-cell transcriptional states and tissue histopathology in a mouse model of Alzheimer's disease.

Abstract: Complex diseases are characterized by spatiotemporal cellular and molecular changes that may be difficult to comprehensively capture. However, understanding the spatiotemporal dynamics underlying pathology can shed light on disease mechanisms and progression. Here we introduce STARmap PLUS, a method that combines high-resolution spatial transcriptomics with protein detection in the same tissue section. As proof of principle, we analyze brain tissues of a mouse model of Alzheimer's disease at 8 and 13 months of age. Our approach provides a comprehensive cellular map of disease progression. It reveals a core-shell structure where disease-associated microglia (DAM) closely contact amyloid-Î² plaques, whereas disease-associated astrocyte-like (DAA-like) cells and oligodendrocyte precursor cells (OPCs) are enriched in the outer shells surrounding the plaque-DAM complex. Hyperphosphorylated tau emerges mainly in excitatory neurons in the CA1 region and correlates with the local enrichment of oligodendrocyte subtypes. The STARmap PLUS method bridges single-cell gene expression profiles with tissue histopathology at subcellular resolution, providing a tool to pinpoint the molecular and cellular changes underlying pathology.

DOI: 10.1038/s41593-022-01251-x

The impact factor: 28.771

Paper 7

Title: Single-nucleus transcriptome analysis reveals dysregulation of angiogenic endothelial cells and neuroprotective glia in Alzheimer's disease.

Abstract: Alzheimer's disease (AD) is the most common form of dementia but has no effective treatment. A comprehensive investigation of cell type-specific responses and cellular heterogeneity in AD is required to provide precise molecular and cellular targets for therapeutic development. Accordingly, we perform single-nucleus transcriptome analysis of 169,496 nuclei from the prefrontal cortical samples of AD patients and normal control (NC) subjects. Differential analysis shows that the cell type-specific transcriptomic changes in AD are associated with the disruption of biological processes including angiogenesis, immune activation, synaptic signaling, and myelination. Subcluster analysis reveals that compared to NC brains, AD brains contain fewer neuroprotective astrocytes and oligodendrocytes. Importantly, our findings show that a subpopulation of angiogenic endothelial cells is induced in the brain in patients with AD. These angiogenic endothelial cells exhibit increased

expression of angiogenic growth factors and their receptors (i.e., EGFL7, FLT1, and VWF) and antigen-presentation machinery (i.e., B2M and HLA-E). This suggests that these endothelial cells contribute to angiogenesis and immune response in AD pathogenesis. Thus, our comprehensive molecular profiling of brain samples from patients with AD reveals previously unknown molecular changes as well as cellular targets that potentially underlie the functional dysregulation of endothelial cells, astrocytes, and oligodendrocytes in AD, providing important insights for therapeutic development.

DOI: 10.1073/pnas.2008762117

The impact factor: 12.779

Paper 8

Title: Deep Multilayer Brain Proteomics Identifies Molecular Networks in Alzheimer's Disease Progression.

Abstract: Alzheimer's disease (AD) displays a long asymptomatic stage before dementia. We characterize AD stage-associated molecular networks by profiling 14,513 proteins and 34,173 phosphosites in the human brain with mass spectrometry, highlighting 173 protein changes in 17 pathways. The altered proteins are validated in two independent cohorts, showing partial RNA dependency. Comparisons of brain tissue and cerebrospinal fluid proteomes reveal biomarker candidates. Combining with 5xFAD mouse analysis, we determine 15 AÎ²-correlated proteins (e.g., MDK, NTN1, SMOC1, SLIT2, and HTRA1). 5xFAD shows a proteomic signature similar to symptomatic AD but exhibits activation of autophagy and interferon response and lacks human-specific deleterious events, such as downregulation of neurotrophic factors and synaptic proteins. Multi-omics integration prioritizes AD-related molecules and pathways, including amyloid cascade, inflammation, complement, WNT signaling, TGF-Î² and BMP signaling, lipid metabolism, iron homeostasis, and membrane transport. Some AÎ²-correlated proteins are colocalized with amyloid plaques. Thus, the multilayer omics approach identifies protein networks during AD progression.

DOI: 10.1016/j.neuron.2019.12.015

The impact factor: 18.688

Paper 9

Title: Cell type-specific changes identified by single-cell transcriptomics in Alzheimer's disease.

Abstract: The rapid advancement of single-cell transcriptomics in neurology has allowed for profiling of post-mortem human brain tissue across multiple diseases. Over the past 3 years, several studies have examined tissue from donors with and without diagnoses of Alzheimer's disease, highlighting key changes in cell type composition and molecular signatures associated with pathology and, in some cases, cognitive decline. Although all of these studies have generated single-cell/nucleus RNA-seq or ATAC-seq data from the full array of major cell classes in the brain, they have each focused on changes in specific cell types. Here, we synthesize the main findings from these studies and

contextualize them in the overall space of large-scale omics studies of Alzheimer's disease. Finally, we touch upon new horizons in the field, in particular advancements in high-resolution spatial interrogation of tissue and multi-modal efforts-and how they are likely to further advance mechanistic and target-selection studies on Alzheimer's disease.

DOI: 10.1186/s13073-022-01136-5

The impact factor: 15.266

Paper 10

Title: Guidelines for bioinformatics of single-cell sequencing data analysis in Alzheimer's disease: review, recommendation, implementation and application.

Abstract: Alzheimer's disease (AD) is the most common form of dementia, characterized by progressive cognitive impairment and neurodegeneration. Extensive clinical and genomic studies have revealed biomarkers, risk factors, pathways, and targets of AD in the past decade. However, the exact molecular basis of AD development and progression remains elusive. The emerging single-cell sequencing technology can potentially provide cell-level insights into the disease. Here we systematically review the state-of-the-art bioinformatics approaches to analyze single-cell sequencing data and their applications to AD in 14 major directions, including 1) quality control and normalization, 2) dimension reduction and feature extraction, 3) cell clustering analysis, 4) cell type inference and annotation, 5) differential expression, 6) trajectory inference, 7) copy number variation analysis, 8) integration of single-cell multi-omics, 9) epigenomic analysis, 10) gene network inference, 11) prioritization of cell subpopulations, 12) integrative analysis of human and mouse sc-RNA-seq data, 13) spatial transcriptomics, andÂ 14) comparison of single cell AD mouse model studies and single cell human AD studies. We also address challenges in using human postmortem and mouse tissues and outline future developments in single cell sequencing data analysis. Importantly, we have implemented our recommended workflow for each major analytic direction and applied them to a large single nucleus RNA-sequencing (snRNA-seq) dataset in AD. Key analytic results are reported while the scripts and the data are shared with the research community through Â GitHub. In summary, this comprehensive review provides insights into various approaches to analyze single cell sequencing data and offers specific guidelines for study design and a variety of analytic directions. The review and the accompanied software tools will serve as a valuable resource for studying cellular and molecular mechanisms of AD, other diseases, or biological systems at the single cell level.

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The impact factor: 18.879