Integrating single-cell transcriptomic data to track and compare microglia's temporal responding of AD and aging process

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Submission date: 2018-12-19

Abstract

As the population ages, Alzheimer's disease (AD) is becoming one of the biggest diseases, while it's now incurable and most clinical trials on it failed in the last 16 years. Lack of comprehensive understanding of the initiation and function of major pathologies like amyloidβ(Aβ) and tau depositing or neuroinflammation contributes to these failures. In this proposal, I chose microglia in hippocampus of model animals as model system to characterize their expressing pattern trying to better recapitulate the temporal immune responses in AD and aging process.

There should be dynamic responsive and nonresponsive, pro-degeneration and anti-degeneration subtypes of microglia cells during AD progress, and they may recurrent or reverse the signatures of aging process. The early transcriptomic features of microglia cells may reflect pathological changes before Aβ aggregation, tau deposition and neuron dysfunction.

To track and compare microglia cells temporal transitions in AD and aging process, I will integrate, analyze and model existing single cell transcriptomic and ChIP-seq data from different mouse models as well as bulk transcriptomic data from middle-aged and aged human. The results of this study are expected to leverage our understanding of the relation of brain's immune system with aging and AD, and provide basic knowledge for researchers to develop effective targeting neurodegenerative stages earlier than Aβ oligomers(AβO) play its detrimental role.

Hypotheses and Aims

While microglia play a complex role in development and aging, and maybe a dual role in neurodegenerating diseases, I hypothesized that microglia are dynamic and heterogenous during different biological process, i.e., there should be dynamic responsive and nonresponsive, pro-degeneration and anti-degeneration subtypes of microglia during AD progress and the temporal transcriptional signatures of those different cells may recurrent or likely reverse the developmental or aging process. I propose to integrate single cell RNA sequencing data from CK-p25 inducible mouse and CK, AD-transgenic and P30, P100, P540 C57BL/6 WT hippocampus to test this hypothesis, to find out which and how the specific sets of gene expression drive the which subtypes of microglia to accelerate or alleviate Aβ oligomer formation and neurocognitive dysfunction and how it relates to aging process.

The aim is to track and compare microglia's temporal responding in aging and AD process in different mouse models. To achieve this, six staged aims are needed:

1. Collecting and controlling quality of single cell RNA seq count matrices of mouse and human brain tissues with AD and their corresponding control.
2. Aligning groups of data sets.
3. Visualizing and quantifying alignment result.
4. Investigating microglial subtypes with metadata, analyzing their cellular components, molecular signatures and specific pathways.
5. Analyzing the related histone markers changes of AD in the promoter or enhancer region of those expression marker genes of AD responsive microglial

subtypes.

6. Compare the resulting AD responsive microglial molecular signatures with previous identified differentially expressed gene sets in aged human and mouse microglia separately.

Background and Significance

Alzheimer's Disease is the most prevailing dementia among the population >65 years old now, and is predicted to affect 50 million people by 2030(Winblad et al., 2016). Unfortunately, now there's no complete molecular pathologies perfectly explaining its happening, not to mention the cure of it. Over 400 clinical trials were run between 2002 and 2018, but only one drug, memantine, was approved(Cummings, 2018).

Several pathological hypotheses have been proposed for AD, including abnormal Aβ aggregating around neurons(Hardy and Higgins), twisted tau protein fibers inside neurons(Mudher and Lovestone), damage of cholinergic neurons(Hasselmo et al., 1991), inflammation (Zotova et al., 2010), oxidative stress(Markesbery, 1997), etc.. However, only cholinergic hypothesis has led to 5 drugs. The failure of anti-amyloid or anti-tau agents in their clinical trials can be ascribed to the lack of comprehensive understanding of the entangled relation of various hypotheses and the exquisite initiation and function mechanism of Aβ fibrils and tau in brain. Phosphorylation of various sites on tau protein is an intracellular process regulated by various pathways and its phenotype is late-staged and irreversible(Neddens et al., 2018). Instead, studies on preclinical AD found that abnormal Aβ42 levels in several core regions of the default mode network already affect brain connectivity(Palmqvist et al., 2017). While directly targeting Aβ plaques failed, identification of agents targeting Aβ receptors on neurons is still popular these years. EVP-6123, PTI-125(Wang et al., 2017) and TTP488(ClinicalTrials.gov Identifier: NCT02080364) are in their Phase II or III clinical trials. 12 molecules were found as potential inhibitors of AβO-LilrB2 interaction (Cao et al., 2018). But those neuronal receptors play a two-sided and not complete role in Aβ pathology. Considering uncertain results from those efforts targeting harmful AβO-neuron interaction, researchers are trying to elucidate signatures before Aβ does harm to neurons and taking a systematic view on how other factors, especially brain's immune system, function in the neurodegeneration process.

Over the last decade, researchers found that as an important component of innate-immune system in the central nervous system (CNS), microglia cells can interact with Aβ before its deposition, maintaining Aβ's homeostasis(Lai and McLaurin, 2012), triggering inflammation(Boza-Serrano et al., 2018)，clean up debris(Poliani et al., 2015) and modulating synaptic function(Wu et al., 2015). And microglial adhesion and migration to Aβ is decreasing with aging(Fang et al., 2018). Thus, elucidation of how microglia responding with the progress of AD is a highly rated way to uncover AD pathology and develop early therapeutics.

In major brain disorders including AD, as elucidated by Expression Weighted Cell Type Enrichment using single cell transcriptomes, major brain cell types including microglia all have pathological changes(Skene and Grant, 2016). Recent single cell

analysis of mouse microglia indicated that microglia are heterogenous cells and only small subsets of them are involved in inflammatory response with brain injury, which have similar Ccl4 changes with aging process.(Hammond et al., 2018). A transcriptomic atlas of aged human microglia has been successfully built this year, in which we found genes primarily expressed by aged microglia are enriched in susceptible genes for AD(Olah et al., 2018). Chromatin Immunoprecipitation(ChIP) and RNA-seq of CK-p25 inducible mouse previously revealed AD-associated genetic variants are enriched in enhancers involving immune processes(Gjoneska et al., 2015), and single cell RNA sequencing(scRNA-seq) of the same mouse model primarily identified early and late response microglia to neurodegeneration(Mathys et al., 2017). Also, in AD-transgenic mouse brains, the inhibitory checkpoint pathways of a unique microglia-type is downregulated in a two-step process during neurodegeneration(Keren-Shaul et al., 2017). But previous transcriptomic analysis of two animal models of AD, Tg2576 Swedish APP and TgCRND8 Swedish plus Indiana APP showed that different mouse models capitulate human AD-associated transcriptional signatures differently(Rothman et al., 2018). Integration of data sets of the same tissues enables 'meta-analysis' to compare and validate results across laboratories and technologies, and can find rare population and new biological facts which are precluded in original analyses(Butler et al., 2018). So to fully understand how immune system, especially microglia functions in AD progress, and how it relates to other pathologies like Aβ in cerebrospinal fluid and tau in neurons and how it associates with aging process, it's crucial to pool existing single-cell transcriptomic data from microglia in different AD mouse models and analysis the cellular types and temporal signatures of them.

Expected results from this project will lay the foundation of early and effective therapy development for AD.

Preliminary Results

I have previously characterized gonocytes' developmental process through single cell transcriptomic analysis and compared in-house data sets with data from (Chen et al., 2018)(unpublished results).

I obtained raw data from accession numbers GSE98969(Tg-AD), GSE103334(CK-p25 model), GSE65159(CK-p25 ChIP), GSE121654(P30, P100, P540 C57BL/6J). Human aging microglia RNA-sequencing dataset is obtained from http://shiny.maths.usyd.edu.au/Ellis/MicrogliaPlots/ and human middle-aged microglia transcriptomic dataset is derived from GSE73721.

Detailed Aims

1. I proposed to use custom scripts to preprocess data and select genes. This will be done by filtering low expressed cells, calculating the dispersion of all genes, selecting 1000 highest dispersive gene lists in each data set and taking the union of the lists. Especially, I will use canonical microglia markers Fcrls, Trem2 and C1qa to select true microglia in each dataset.
2. I proposed to use CCA to define a shared correlation space of the two single cell transcriptome data sets. This will be done by using runCCA function in R

package seruat v3(Stuart et al., 2018).

3. I proposed to use PCA/CCA to identify rare non-overlapping microglia subtypes. This will be done by using CalcVarExpRatio and SubsetData function in R package seruat v3.
4. I proposed to use dynamic time warping to align correlated subspaces. After alignment, I will cluster cells using smart local moving (SLM) algorithm. i.e., cell-cell distance matrixes will be constructed on previously selected aligned canonical correlation vectors, then shared-nearest neighbor graph based on the distance matrix will be constructed and implemented as input to the SLM algorithm. For visualizing, I will use t-distributed stochastic neighbor embedding(t-SNE) algorithm from R package seruat v3. I will build trajectory and investigate cellular component and molecular signatures using monocle, edgeR and clusterProfiler. To find differential expressed genes in each cluster, I proposed to use logFC>2 or logFC<-2, adjusted p-value < 0.05 as threshold.
5. After interesting expressing features are found out, I propose to follow the ENCODE histone ChIP-seq data processing pipeline to analysis the regulation mechanism of those interesting expressing variations from epigenomic viewpoints. More specifically, as shown in fig1, I prepare to use BWA to map reads onto mm10 reference genome and using samtools and picard to get proper alignment file for further peak calling process. MACS will be used to call peak and BEDTools to generate signals and customize scripts to find changes(fig2).

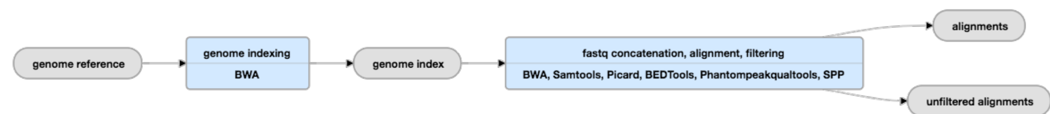

Fig1. Pipeline for mapping of FASTQs

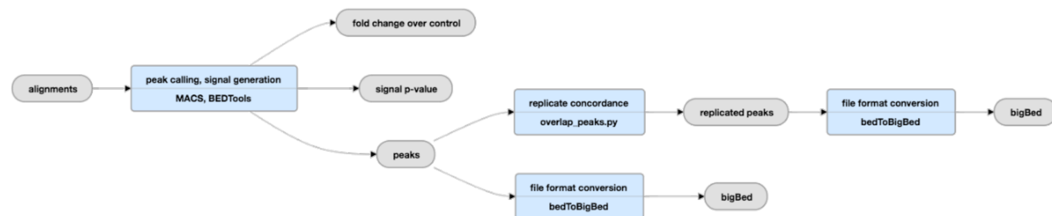

Fig2. Pipeline for histone ChIP-seq peak calling

6. To test each resulting differentially expressed gene sets reflecting pro and anti-AD signatures' enrichment in human aged microglia gene set, I should first find all of the homologous subsets of genes between mouse and human and test with and over-representation analysis.

Anticipated Results

I anticipated that in each data sets, there are small percentage of contaminating cells (including neurons, endothelial cells, etc.) which can be characterized using a first round independent component analysis- based clustering.

After removing contaminating cells, I anticipated to see cell clusters with different ages and AD progress. It's possible that most disease cells cannot be distinguished from cells with heathy states, with one or two unique AD responsive subsets changing over the different stages of AD. Cell types never be defined before may also be found by analyzing the integrated single cell transcriptomic data.

I anticipated that of all the microglia in each process or state (healthy young and old, AD pathological state), some of the clusters should be immune cell-like, upregulating expressing of those genes enriched in immune response pathways (e.g., Ccl3, Ccl2, Ccl7, Ccl9, Ccl12, Il1b, Tnf). Some of the clusters in each state will show neurotoxic or pathological potential, indicating there are dynamic 'good' or 'bad' microglia.

Of those changing clusters between different healthy or degenerative stages, there should be increasing and shrinking ones. I anticipated the distinct molecular markers they express significant pro or anti-AD process, which we can add up to our potential therapeutic targets' list.

There may (not for sure) be some overlap with aging and AD responsive cells. When there's no common cellular component, I anticipated there should be similar or opposite molecular features between the two process. If so, such results can give us clues on how aging influence brain's immunity and how it relates to AD pathology.

Of those interesting cell types, their unique expression pattern may be driven by different epigenetic modifications. Using existing ChIP-seq data, I anticipated to find out the changing cis-regulating element of those markers in AD process, e.g., transient, consistent or late H3K4me3 increase/decrease in promoter regions and transient, consistent or late H3K27ac increase/decrease in enhancer region, which will further illustrate the mechanism under AD development.

Alternative methods like scmap or mnnCorrect are needed if the proposed computational methods fall through. To further validate results from the integration of public data, single molecular FISH and immunohistochemical analysis on mouse model are needed. Also, to reinforce the biological and medical significance of this project, co-staining with antibody which anti the molecular markers found in each functional microglia cell type on human hippocampus sample from age-matched control and AD samples is welcome.

To conclude, this study's cluster results will leverage our understanding of microglia types. And It's hopeful to find the responsive(both pro and anti-AD) subtypes, whose molecular signatures may reflect their function and relation with Aβ deposition in different disease stages. The enrichment of these signature genes in human aged microglia specific genes will shed light on the dynamic relationship between AD and aging. Of several AD and aging related gene sets, their promoter or enhancer's histone markers' changes in AD will show the epigenetic regulation of those featured genes.

All these results will lead to better understanding of immunological response in AD and help therapeutic design targeting any essential component of AD progress, especially pathological process before Aβ deposits.

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