

Single-Nucleus RNA Sequencing Reveals Dysregulation of FOXO1 and PPARγ in Microglia Associated with Alzheimer’s Disease

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Hyojoon Kim¹, Jaejoon Kim¹, Shari Atilano², Satish Patnaik²
¹OCSA, Santa Ana, CA, USA, ²UC Irvine, Irvne, CA, USA

Progressive imbalance of the FOXO1–PPARγ axis in disease-associated microglia (DAM) is associated with Alzheimer's disease pathology. Decreased FOXO1 and sustained PPARγ over-expression promote lipid droplet accumulation and impair chronic phagocytic function, accelerating neurodegeneration through disrupted lipid metabolism and reduced debris clearance.

INTRODUCTION

Microglia, the resident immune cells of the central nervous system, maintain lipid homeostasis by clearing cellular debris and damaged membranes. A specialized microglial population, termed disease-associated microglia (**DAM**), accumulates around amyloid plaques and contributes to plaque containment. However, during aging and neurodegeneration, **DAM** progressively lose homeostatic functions, including efficient autophagy and phagocytosis.

Recent studies have identified lipid-droplet-accumulating microglia as a hallmark of aging and neurodegenerative diseases, including AD. These cells exhibit impaired phagocytosis, elevated reactive oxygen species production, and increased pro-inflammatory cytokine secretion, suggesting that disrupted lipid handling contributes to microglial dysfunction.

Peroxisome proliferator-activated receptor gamma (**PPARγ**) is a key regulator of lipid metabolism and inflammation. In microglia, PPARγ promotes fatty acid uptake, lipid droplet formation, and phagocytic activity. Although PPARγ agonists reduce amyloid burden, tau pathology, and neuroinflammation in experimental models, increased PPARγ expression has also been reported in AD brains, raising questions regarding its role in disease progression.

Forkhead box O1 (**FOXO1**) negatively regulates PPARγ activity and plays an important role in metabolic homeostasis. Dysregulation of the FOXO1–PPARγ axis has been implicated in metabolic disorders, yet its contribution to AD pathogenesis remains unknown.

Single-nucleus RNA sequencing (**snRNA-seq**) provides a powerful platform for dissecting cell-type-specific transcriptional changes in the human brain and offers an opportunity to investigate the **FOXO1–PPARγ** regulatory network in AD-associated microglia.

PURPOSE & METHODS

Purpose

If dysregulation of the FOXO1–PPARγ axis occurs in microglia, it may be associated with pathological processes in Alzheimer’s disease because it intersects with lipid metabolism in microglial cells, potentially altering phagocytic functions and thus worsening the progression of Alzheimer’s disease.

Materials & Methods

snRNA-seq Data : GSE174367

- AD(n = 11), Control(n = 7)
- Female(n = 8), Male(n = 10)
- 74–90+ years old
- Total Cell : 61472(AD: 38676, Control : 22796)
- Total Microglial Cell : 4126(AD: 2742, Control : 1384)
- Plaque Stage
 - None(5781)→Stage A(9627)→Stage B(23700)→Stage C(22364)
 - 1) Control : None(5781), Stage A(9627), Stage B(7388)
 - 2) AD : Stage B(16312), Stage C(22364)

Analysis of snRNA-seq data

- R language(ver 4.5.2) by R studio Seurat (ver 5.4.0)**
- Packages**
 - Analysis(Seurat, SeuratObject),
 - Visualization(dplyr, future, ggplot2, hdf5r, sp, tibble, tidy)
- Statistical analysis**
 - wilcox.test(Wilcoxon rank sum test with continuity correction)
 - kruskal.test(Kruskal-Wallis rank sum test)

RESULTS

A Mean Expression: AD vs Control

B Total Microglial Cells

C Homeostatic

D DAM

Fig1. umap of total microglial cells(A) and AD vs Control(B). Microglial cell population ratio of AD and Control(C)

Fig2. Gene expression heatmap of AD and Control(A), Violinplot of FOXO1 and PPARγ(Wilcoxon rank sum test) (B: Total, C: Homeostatic microglial cell, D: DAM)

Fig3. umap of total microglial cells by plaque stage(A) Lineplot of FOXO1 and PPARγ by plaque stage(B) (Kruskal-Wallis rank sum test)

Fig4. Lineplot of FOXO1, PPARγ and Phagocytosis gene(TREM2, TYROBP) by plaque stage (Wilcoxon rank sum test)

Fig5. umap of total microglial cells by plaque stage(A) Lineplot of FOXO1 and PPARγ by plaque stage(B) (Kruskal-Wallis rank sum test)

DISCUSSION & CONCLUSIONS

Discussion

- This study utilized snRNA-seq data and compared AD and control groups to identify specific pathological gene expression patterns in AD. As shown in umap, each cell exhibited a distinctive cluster, supporting the reliability of the data.
- Dysregulation of FOXO1 and PPARγ has been observed in diseases associated with abnormal lipid metabolism, such as atherosclerosis, and has led to various pathological mechanisms. This dysregulation was observed in microglial cells and DAM in particular from Alzheimer's disease (p<0.001), a finding reported for the first time.
- As Alzheimer disease progressed, FOXO1 decreased, while PPARγ increased in DAM (p<0.001). This pattern is consistent with the mechanisms underlying their inhibitory interaction revealed in previous studies.
- As AD progresses, PPARγ increases in DAM, whereas phagocytosis-related genes show a different pattern: increased in the early phase but decreased in the late phase. While PPARγ is generally known to increase phagocytosis, some studies have reported that lipid droplet-rich microglia from aged mice showed impaired phagocytosis compared to cells without droplets. Therefore, our results suggest that sustained PPARγ overexpression in AD would lead to a decline in phagocytosis function in DAM and promote lipid accumulation, ultimately contributing to disease progression.
- These data shift the perspective from a binary “protective vs detrimental” view of PPARγ toward a model in which sustained PPARγ activation suppresses FOXO1-dependent homeostatic programs and contributes to microglial dysfunction. Although PPARγ agonists demonstrated anti-inflammatory and modest plaque-reducing effects in preclinical animal models, large clinical trials failed to show significant cognitive benefit in Alzheimer’s disease. Emerging transcriptomic data suggest that chronic PPARγ activation by reduced FOXO1 expression may reinforce lipid-accumulating, functionally impaired microglial states, particularly in established disease, highlighting the need for stage- and cell-type-specific therapeutic strategies.

Conclusion

This stage-dependent imbalance within the FOXO1–PPARγ axis provides a mechanistic framework for interpreting the context-specific effects of PPARγ in neurodegeneration. Therapeutic strategies may therefore require selective modulation that restores FOXO1-dependent homeostatic programs without exacerbating chronic PPARγ-driven lipid accumulation.

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CONTACT

Hyojoon Kim
henryk7@hs.uci.edu

Shari Atilano
satilano@hs.uci.edu