

# Analyzing single-cell transcriptome sequencing data reveals immunological mechanisms of microglia-associated diseases

**Yida Wang**

School of Public Health, Binzhou medical university, Yantai, Shandong, 264003, China

Jessiess2023@outlook.com

**Abstract.** In recent years, researchers have increasingly focused on the role of microglia in various neurological disorders, such as multiple sclerosis (MS). The single-cell transcriptome sequencing technology has provided new opportunities to unravel the roles and immunological mechanisms of microglia in a variety of neurological diseases. In this paper, the author explored the mechanisms of microglia in diseases of central nervous system, such as MS by analyzing single-cell transcriptome sequencing data with R. The authors investigated the regulation of microglia activation by signaling pathways, the role of microglia in the pathogenesis of brain dysfunction. Data analysis by single-cell transcriptome sequencing provides new perspectives and understanding of the pathophysiology of related diseases, and new ideas and strategies for their treatment and prevention.

**Keywords:** single-cell transcriptome sequencing, microglia, immunologic mechanisms.

## 1. Introduction

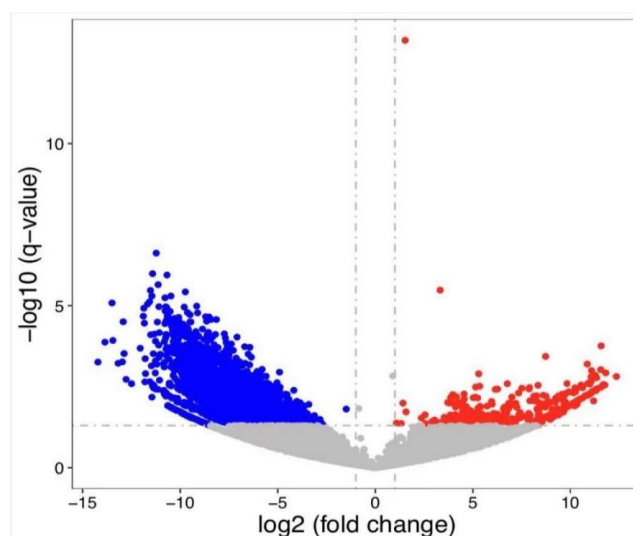
Neurological disorders such as multiple sclerosis (MS), have serious impacts on human health and life quality of patients, which are associated with the dysfunctions of microglia as the important immune cells in the brain. The advances in single-cell transcriptome sequencing technology, which enables sequencing of total RNA at the single-cell level and helps to reveal cellular heterogeneity, trace cells origin, and analyze cellular transformation profiles, providing new opportunities to reveal the role of microglia in a variety of neurological disorders and the immunological mechanisms. This paper provided insights into the functional changes of microglia in these diseases and the associated immunological mechanisms by analyzing single-cell transcriptome sequencing data with R. The author analyzed the abnormalities and differential expression genes of microglia in diseases of central nervous system, such as MS, and focused on the signaling pathways in regulation of microglia activation, as well as the mechanisms by which microglia regulated brain homeostasis and resulted in brain dysfunction. Analysis the data of single-cell transcriptome sequencing provides new perspectives and understanding of the pathophysiology of related diseases, and new ideas and strategies for treatment and prevention.

## 2. Multiple sclerosis (MS)

MS is a chronic disorders in brain associated autoimmune dysfunctions, leading to demyelination and neurodegenerative lesions. Studies have shown that microglia, a subset of innate immune cells,

involved in the development of MS through regulation of brain homeostasis, such as HLA-DR mediated lesions [1].

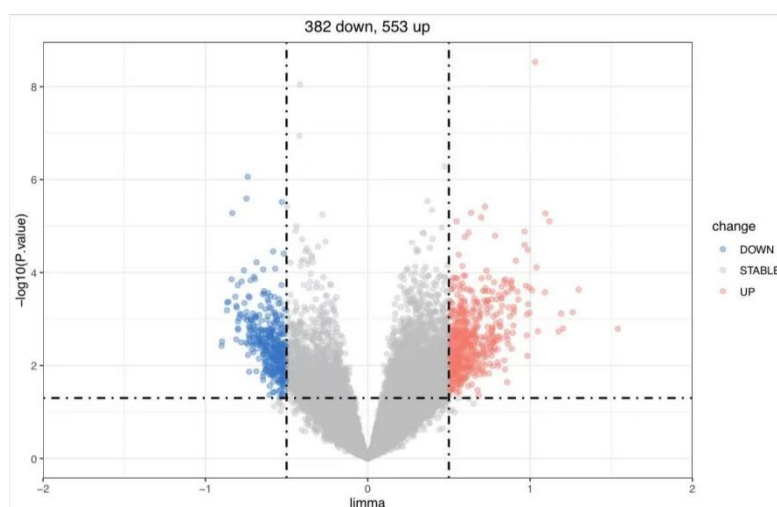
At the site of the lesion, microglia are activated and release large amounts of inflammatory factors, leading to neuronal damage and death. However, recent studies have found that it is possible to inhibit the inflammatory process and protect neurons from damage through regulating activation of microglia, providing new possibilities for the treatment of MS. By comparing microglia transcriptome data (GSE234700) from healthy populations and MS patients, a series of differential gene expression related to inflammation regulation was identified, with significantly up-regulated genes of *ND6* and *MTATP6PI*, as well as significantly down-regulated genes of *IL33*, *DAGLB* and *C12orf4*. Among them, the up-regulation of mitochondria-related gene *MTATP6* was able to affect premature neurodegeneration (Figure 1), which was similar to previous reports [2].



**Figure 1.** The volcano plot of differential expression genes in MS.

### 3. Alzheimer's disease (AD)

AD is a chronic neurodegenerative disease with the microglia dysfunctions. Physiologically, microglia remove  $\beta$ -amyloid proteins to maintain the brain homeostasis through phagocytosis. However, for AD patients, microglia phagocytosis is impaired, leading to the accumulation of  $\beta$ -amyloid plaques, which in turn triggers neuroinflammation and neuronal death. The pathogenic genetic variants further suggests the relationship of microglia to AD development. Therefore, restoration of normal function of microglia may be an effective means of treating AD. Approximately half of the AD risk loci are directly associated with microglia and neuroinflammation from GWAS studies and Meta-analysis [3], such as risk genes encoding APOE and the triggering TREM2. In addition to this, genetic variants were found in genes that are predominantly or exclusively expressed in myeloid cells as the strongest risk genes for AD, highlighting the importance of microglia in the brain microenvironment [4]. Here, a series of differentially expressed genes were identified by comparing microglia transcriptome data (GSE243243) from healthy populations and AD patients. For example, the expression of genes *RNF144B*, *ZNF83*, *NAPEPLD*, *RRAGB*, and *SIRPB2* was significantly up-regulated whereas the expression of genes *RBM17*, *RSRC1*, *TOP1*, *PROC*, and *TMEM52B* was significantly down-regulated. It is noteworthy that the expression of trigger receptor encoding APOE and TREM2 was not significant differentially expression (Figure 2). The gene of topoisomerase 1 (TOP1) validated previous studies [5], suggesting that neurodegeneration could be partially blocked by supplementation of NAD with nicotinamide riboside and that p53 deletion partially reversed neurodegeneration in TOP1 cKO mice.

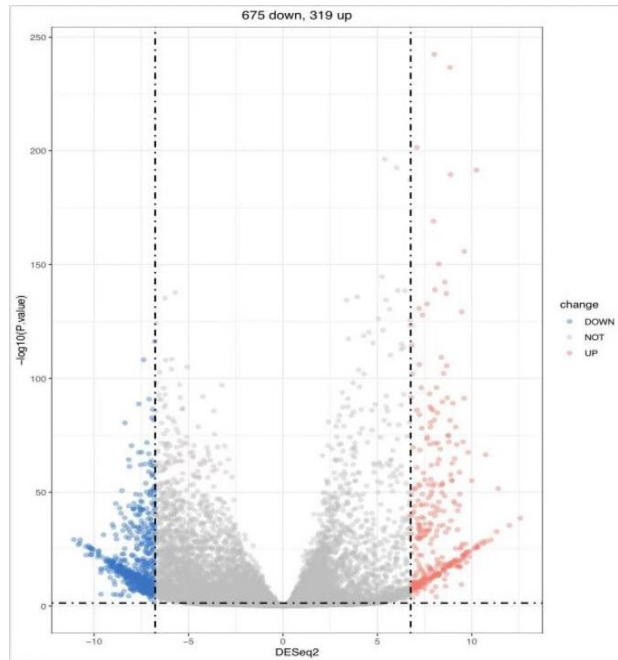


**Figure 2.** The volcano plot of differential expression genes in AD.

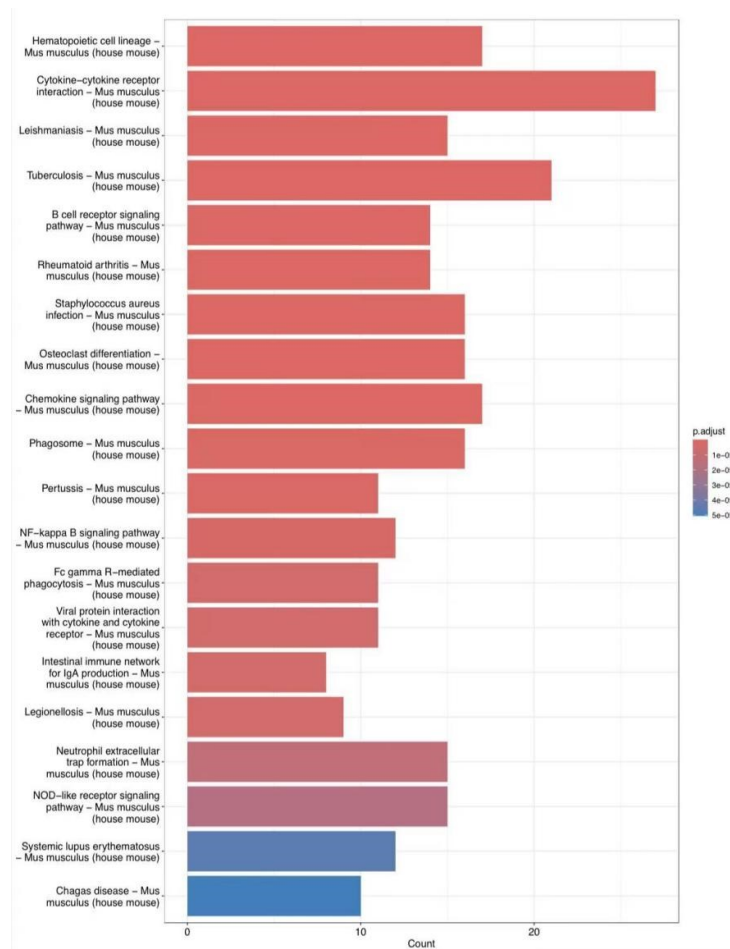
#### 4. Traumatic brain injury (TBI)

TBI is neurological disorder with a pathologic process that includes both primary and secondary injury. In acute phase, trauma causes primary injury through triggering inflammation, apoptosis, and oxidative stress, resulting in brain parenchyma in 24 hours. Many cells involved in the pathogenesis such as microglia, oligodendrocytes and their precursor cells, and astrocytes. Following CNS injury, microglia are rapidly activated to a hypertrophic amoeboid form, whereby reactive microglia proliferate, polarize, and enter into injury sites. Recent studies have also shown that NG2 glial cells, exhibit rapid cellular changes after injury, similar to microglia. In addition, astrocytes also respond to injury and begin to proliferation with changes in their morphology, gene expression and functions. In chronic phase, neuroinflammatory response triggered by pro-inflammatory microglia (M1), reactive astrocyte crosstalk, leading to secondary behavioral and cognitive dysfunctions [6].

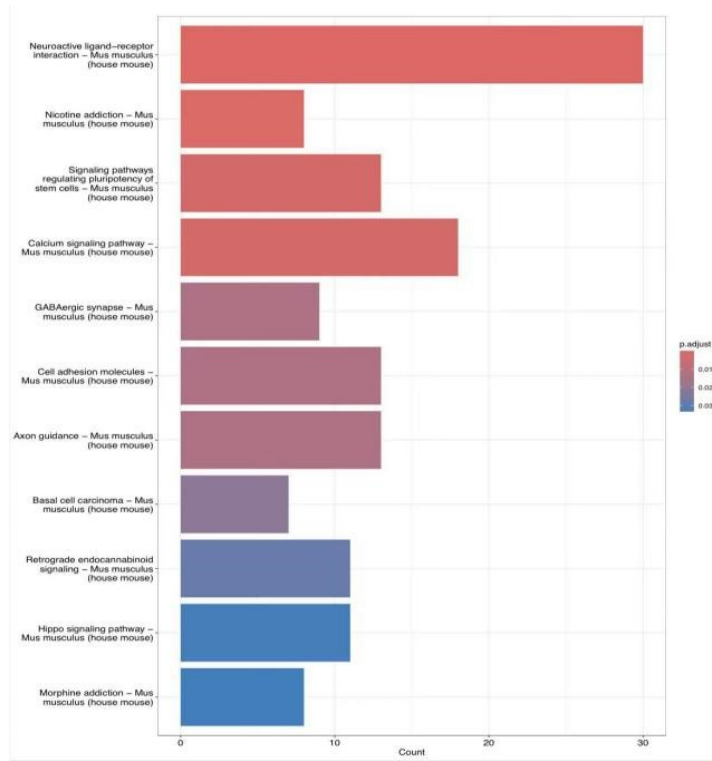
Secondary injury is mainly caused by factors such as inflammatory response and oxidative stress. Studies have shown that the initial pathological mechanisms of microglia-mediated TBI is mainly that microglia promote neuronal regeneration and repair by releasing inflammatory factors and chemokines. However, as the injury worsens, overactivation of microglia may lead to neuronal death and tissue necrosis [7]. Therefore, regulating the activity of microglia and balancing their pro-inflammatory and anti-inflammatory effects may be the key to treating traumatic brain injury. Here, differential genes regulating the highly coordinated response of microglia were revealed by comparing microglia transcriptome data (GSE167459) from healthy populations and TBI patients (Figure 3). In addition, upregulation of the chemokine signaling pathway and the NF-Kappa B pathway were found to promote microglia responses by KEGG analysis. In addition, microglia regulate physiological activity through signalling pathway such as ER  $Ca^{2+}$  release, and increasing the number of receptors or injecting stimulants modulates microglia motility (Figure 4). Downregulation of signaling pathway regulating pluripotency of stem cells and calcium signaling pathway may attenuate microglia reactivity (Figure 5). The regulation of leukocyte proliferation and immune effects was found to upregulate microglia activity by GO analysis (Figure 6) while the signaling pathways related to membrane potential regulation and pattern designation process could downregulate microglia activity (Figure 7).



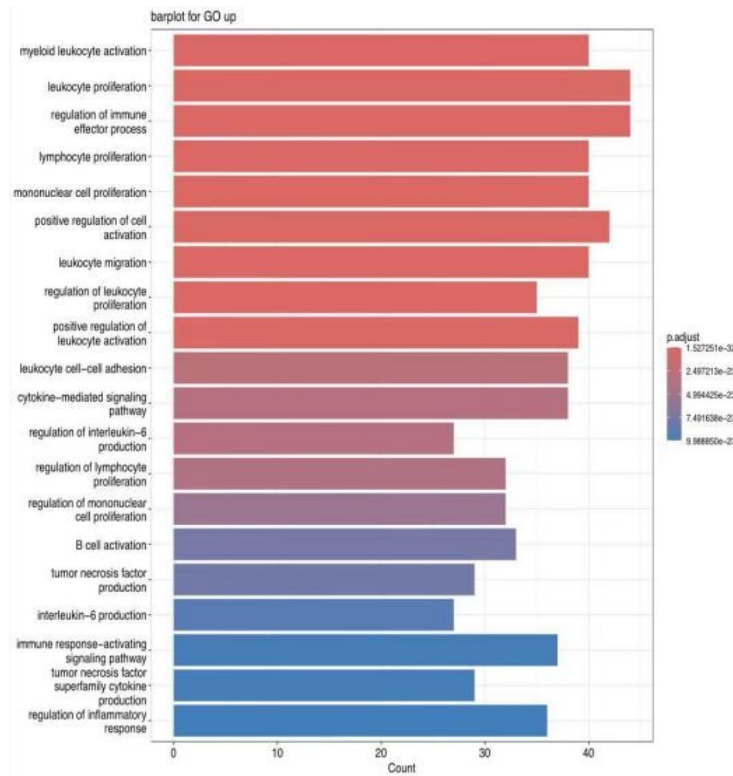
**Figure 3.** The volcano plot of differential expression genes in TBI.



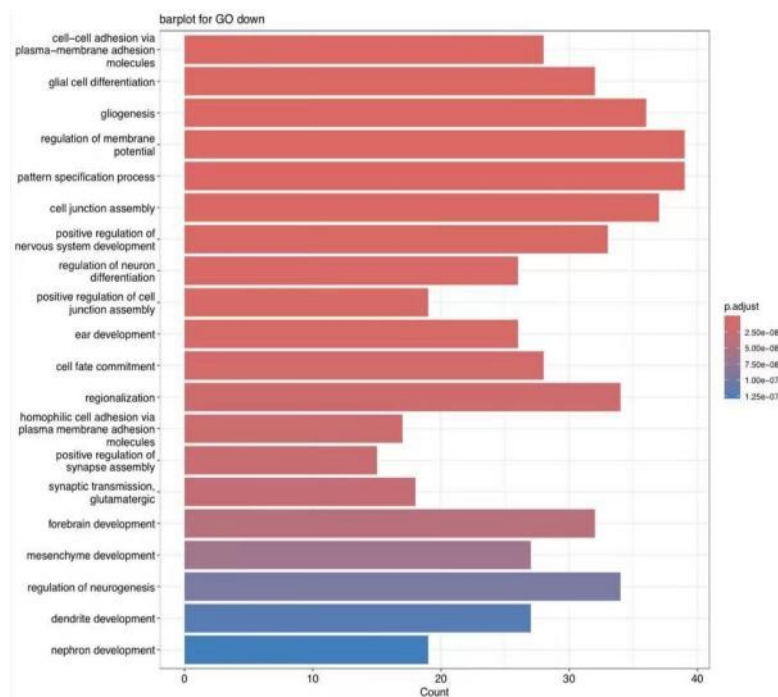
**Figure 4.** The KEGG pathways in TBI (up-regulated genes).



**Figure 5.** The KEGG pathways in TBI (down-regulated genes).



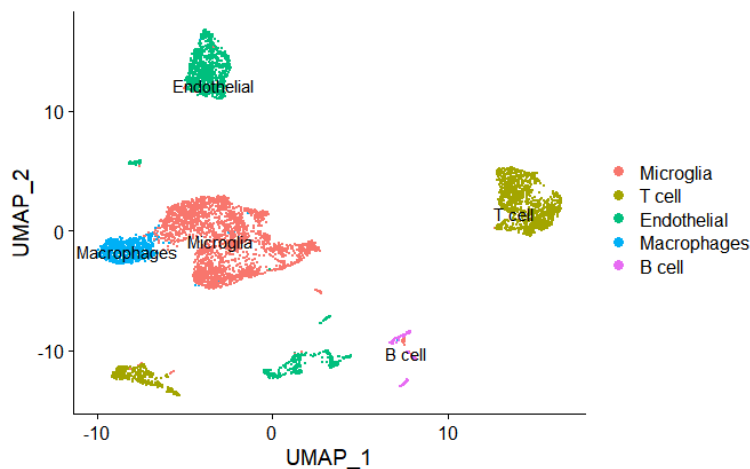
**Figure 6.** The GO pathways in TBI (up-regulated genes).



**Figure 7.** The GO pathways in TBI (down-regulated genes).

### 5. Glioblastoma (GB)

GB is a type of primary brain tumor involved with enhanced angiogenesis. The microglia are macrophage-like cells, which have been shown to be homologous to brain microglia and BMDM and confirmed to contribute to GB progression through regulation immune system and tumor microenvironment [8]. Macrophages with anti-tumor phenotype have phagocytic, antigen-presenting, and cytotoxic characteristics, and mainly clear tumor cells by enhancing the action of cytotoxic T cells. In addition, macrophages with anti-inflammatory and pro-tumor phenotypes associated with tissue repair promote tumor growth and invasion by participating in processes such as tissue remodeling, angiogenesis, and anti-inflammatory cytokines [9]. Here, by analyzing microglia transcriptome data from GB patients (GSE202371), changes in the ratio of glial cells to immune cells in the tumor group of patients were found by dimensionality-decreasing clustering (Figure 8), suggesting that an imbalance in immune homeostasis is responsible for tumor progression.



**Figure 8.** The UMAP of cellular dimensionality reduction clustering in GB.

## 6. Conclusions and outlook

Brain homeostasis is important for maintaining the normal function through regulation of microglia as specialized immune cells in the brain, removing harmful substances, inhibiting inflammatory responses, and maintaining neuronal metabolic balance. However, when microglia function abnormally, it may lead to imbalance of brain homeostasis, which in turn may cause a variety of neurological diseases. Therefore, an in-depth study of the mechanisms of microglia's roles in brain homeostasis are important for understanding and treating neurological diseases.

By analyzing single-cell transcriptome sequencing data with R, the author delved into the role and immunological mechanisms of microglia in neurological diseases. This study provides new perspectives and understanding of the pathophysiology of related diseases, and new ideas and strategies for the treatment and prevention of related diseases. This study not only expands the understanding of microglia in a variety of neurological diseases, but also provides an important reference for future in-depth studies of the mechanisms and treatment of neurological diseases.

The study of microglia is still in its infancy, and many mechanisms have not yet been clarified. In the future, it's need to further study the biological properties and functional mechanisms of microglia, with a view to providing new ideas and methods for the in-depth understanding and effective treatment of neurological diseases. At the same time, it's necessary to focus on the potential of microglia in nerve regeneration and repair, with a view to laying the foundation for their widespread promotion in clinical applications.

In conclusion, methods to improve AD were discussed, and neurodegeneration can be partially blocked by supplementation of NAD with nicotinamide riboside, how NAD alters microglial cell activity and blocks neurodegeneration remains to be explored. In addition to this, how MSp53 deficiency reverses neurodegeneration and thus improves AD deserves further developmental studies.

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