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REVIEW ARTICLE

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Microglia Heterogeneity: A Single-cell Concerto

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ABSTRACT

Microglia are tissue-resident macrophages of the central nervous system (CNS) that play crucialroles in development, homeostasis, and response to perturbation. Microglia react to the surrounding environment in a context-dependent manner. However, research on microglial heterogeneity is limited, given the lack of high-resolution and high-sensitivity methods. Recent studies have demonstrated the heterogeneity of microglia on a spatial-temporal scale, benefiting from the advancement of single-cell technologies. Here, we review the current knowledge about microglial diversity during physiological and pathological conditions in humans and mice.

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1. Introduction

Microglia are innate immune cells widely distributed in the brain and can rapidly respond to pathological damage by interacting with other cells (Colonna & Butovsky, 2017; Ransohoff & Perry, 2009). Microglia represent approximately 0.5–16.6% of the total cells in the central nervous system (CNS), depending on the species and anatomical region (Lawson et al., 1990; Mittelbronn et al., 2001). Microglia originate from early erythromyeloid progenitors in the embryonic yolk sac (Gomez Perdiguero et al., 2015; Kierdorf et al., 2013). They undergo lifetime local self-renewal and slow proliferation associated with apoptosis, which makes the embryonic microglial population to further expand during development together with the CNS (Schulz et al., 2012) and support their maintenance across the adult lifespan (Ladeby et al., 2005), with no contribution from blood-derived cells (Askew et al., 2017).

As the first responder to stimuli in the CNS, microglia constantly monitor their territory to maintain stable neurological function. In the past, this distinction has relied heavily on analyzing the density, morphology, surface expression of immune molecules, and electrophysiological characterizations (De Biase et al., 2017; Schmid et al., 2002). Given that microglia play an essential role in the defense and maintenance of CNS homeostasis and other functions, such as directing neuronal development, neurotrophic marker secretion, clearing dying cells, and synaptic remodeling (Butovsky & Weiner, 2018) (Fig. 1), we proposed the hypothesis that microglial heterogeneity contributes to an extensive range

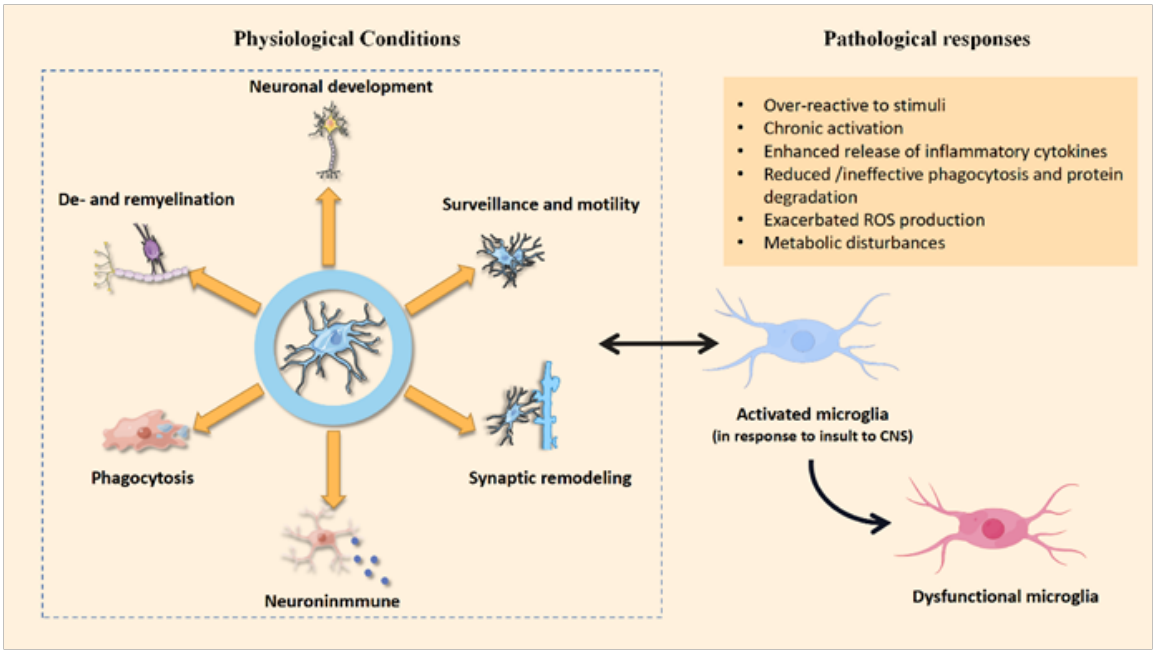

Figure 1. Essential Physiological Functions of Microglia. Under steady-state conditions, microglia execute functions of parenchymal surveillance through process motility. Microglia can interact with neurons and other cells; they also play an essential role in synaptic remodeling, demyelination/remyelination, and neuronal development. In pathological conditions, microglia perform dysfunctional phenotype, which ultimately becomes deleterious.

of functions and responses under physiological conditions.

Over the past decade, advances in single-cell RNA sequencing (scRNA-seq) and lineage tracing have helped reveal the heterogeneity of microglia across different brain regions, development stages, homeostasis, and disease state at a single-cell level. Thus, the regional heterogeneity of microglia has been investigated. Recent studies have comprehensively described microglial differentiation from the embryonic stage to adulthood. Moreover, little is known about the context-dependency of microglial subtypes during disease. Here, we summarize the recent research on single-cell profiles of microglia in the healthy and diseased brain (Table 1). More single-cell analyses are needed to elucidate the molecular signature of human microglia, which could be helpful in the identification of new markers and pathways associated with their physiological functions throughout development, physiological status, and pathophysiological status. This review provides a comprehensive overview of microglial heterogeneity studied using advanced single-cell technologies. We consider that our review contributes to a deeper understanding of the heterogeneity of microglia during development, homeostasis, and disease in humans and mice.

2. Microglia heterogeneity during development and homeostasis in humans and mice

2.1 Microglia diversity during development

Microglial precursors, which originate from erythromyeloid progenitors in the mammalian yolk sac, colonize the brain early in prenatal life and self-renew throughout the lifespan (Askew et al., 2017; Réu et al., 2017). Recent studies have provided a high-resolution view of the transcriptional landscape of microglial subtypes in the mouse CNS during development (Keren-Shaul et al., 2017; Li et al., 2019; Masuda et al., 2019; Matcovitch-Natan et al., 2016).

Jordão et al. (2019) identified a distinct microglial group in the mouse embryonic brain that highly expresses membrane-spanning 4-domains (MS4A) family members such as MS4A7. However, other recent studies could not identify the MS4A7^{+} microglia subset in the brains of mouse embryos (Li et al., 2019; Masuda et al., 2019), possibly because of the different sequencing

	Non-Pathological Tissue	Brain Tumor	Aged Brain	MS	ALS	AD	PD	MDD	LPS Injection	Species	Technologies
Keren-Shaul et al., 2017	■		■		■	■				Mouse	scRNA-seq
Masuda et al., 2019	■			■						Human	snRNA-seq
Lopes et al., 2022	■					■	■	■		Human	scRNA-seq
Zhou et al., 2020	■					■				Mouse/ Human	snRNA-seq
Olah et al., 2020	■					■				Human	scRNA-seq
Smith et al., 2022	■					■				Human	snRNA-seq
Smajić et al., 2021	■						■			Human	scRNA-seq
Mastroeni et al., 2018	■					■	■			Human	scRNA-seq
Borst and Prinz, 2020	■			■						Mouse	scRNA-seq
Liu et al., 2020	■				■					Mouse	scRNA-seq
Sousa et al., 2018	■								■	Mouse	scRNA-seq;CyTOF
Gosselin et al., 2017	■	■								Human	ATAC-seq;ChIP-seq
Venteicher et al., 2017		■								Human	scRNA-seq
Sankowski et al., 2019	■	■	■							Human	scRNA-seq;CyTOF
Ochocka et al., 2021	■	■								Mouse	scRNA-seq
Friebel et al., 2020	■	■								Mouse/ Human	CyTOF
Darmanis et al., 2017		■								Human	scRNA-seq
Böttcher et al., 2020	■							■		Human	CyTOF

Table 1. Research on single-cell profiles of microglia in the healthy and diseased brain. Gray squares marked microglia from a certain condition.

technologies and clustering methods used in those studies. Moreover, it is unclear whether this subset gives rise to CNS-associated macrophages (CAMs) or further differentiates into mature microglia (Jordão et al., 2019; Li et al., 2019; Masuda et al., 2019). A unique microglial subset is characterized by the high expression of genes associated with glycolysis and inflammation, such as *Ldha* and *Mif* (Hammond et al., 2019; Plemel et al., 2020). The overlap between embryonic microglia and inflammation may be due to the microglial functions of synaptic pruning and dead cell clearing, which may be the underlying microglial transcriptomic signature. Additionally, another microglial subpopulation with high expression of apolipoprotein E (*Apoe*), *Ctsb*, or *Tmsb4x* has also been identified (Masuda et al., 2019). Several genes associated with lysosomal functionality (such as *Lamp1*) are expressed at a high level in the $Ctsb^{+}$ microglia subset, which may be involved in its elevated phagocytic activity, suggesting enhanced lysosomal activity in this subclass (Masuda et al., 2019).

Similarly, using scRNA-seq, a microglia subpopulation characterized by high expression of *Clec7a*, glycoprotein non-metastatic melanoma protein B (*Gpnmb*), secreted phosphoprotein 1 (*Spp1*), *Lgals3*, and *Igf1* and having an amoeboid morphology has been found in white matter regions in early postnatal brain regions (Hambardzumyan et al., 2016; Hammond et al., 2019; Li et al., 2019). In the studies mentioned above, Hammond et al. (2019) identified a microglial population enriched in the developing axonal tracts, which are known as axonal tract microglia (ATM). These ATMs were also described using different expression levels of genes associated with lysosomal activation, indicating the potential role of ATMs in pruning myelin sheaths during development (Hughes & Appel, 2020). Li et al. (2019) identified this unique microglial subclass and was termed proliferative-region-associated microglia (PAM). Genes related to PAMs enable the engulfment of newly formed oligodendrocytes, which causes significant cell death at the onset of CNS myelination and may play an essential role in the clearance of overproduced oligodendrocytes (Barres et al., 1992). The PAM subpopulation is synced with the onset of myelination and thus may play a vital role in clearing overproduced oligodendrocytes (Barres & Raff, 1999). Beyond lysosomal acidification-associated genes, the expression of those related to lipid metabolism and the transport was also upregulated, possibly helping the executive function of phagocytosis of lipids rich in oligodendrocytes.

Recent scRNA-seq analysis has revealed that microglia become more homogeneous with fewer phenotypes (Lopes et al., 2022; Masuda et al., 2019; Matcovitch-Natan et al., 2016). Limited regional specialization of microglia across different brain regions in adulthood has also been reported. Adult homeostatic microglia are characterized by genes such as *Fcrls*, *Selplg1*, *C1qa*, and transmembrane protein 119 (*Tmem119*) (Butovsky et al., 2014; Gerrits et al., 2020). However, some typical microglial markers, such as P2Y12 purinoceptor (*P2ry12*) and CX3C chemokine receptor 1 (*Cx3cr1*), do not appear to be uniformly expressed across all homeostatic populations (Hammond et al., 2019).

Specific microglial subpopulations have been confirmed in neurodegenerative diseases correlated with age, such as Alzheimer's disease (AD) (Krasemann et al., 2017), and it is obvious that microglia tend to be more heterogeneous with aging. ScRNA-seq studies have provided more profound insights into the role of aging in microglial heterogeneity. Overall, age-related changes in microglial populations occur and induce an immunogenic phenotype with inflammatory and interferon-responsive profiles (Grabert et al., 2016; Krasemann et al., 2017). These age-associated microglial populations are characterized by high expression of chemokines *Ccl3* and *Ccl4*, *Cst7*, lipoprotein lipase (*Lpl*), genes related to *Ifitm4*, *Ifit3*, *Irf7*, and *Rtp4*. Interestingly, downregulation of the expression of *C2*, *P2RY12*, and *P2RY13*—the key players in microglia-neuron interactions, as well as of genes related to age-related diseases, such as *MS4A4A*, membrane-spanning 4-domains A6A (*MS4A6A*), *BST1*, and *P2RY12*, was reported in a recent study (Lopes et al., 2022). The inflammatory subpopulations may lead to age-related progressive neurodegeneration (Flowers et al., 2017; Young et al., 2021). Overall, microglia are highly heterogeneous at the embryonic and early postnatal phases. Some novel microglia subtypes have been identified, and there exists limited regional specialization of microglia across different brain regions in adulthood. In addition, the heterogeneity of microglia relatively increases during aging (Fig. 2).

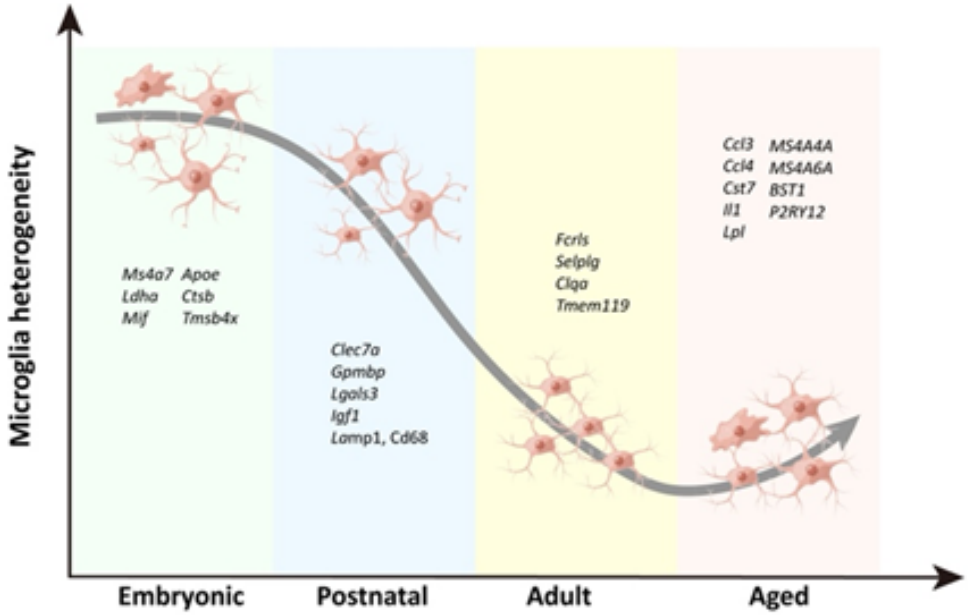

Figure 2. Microglia heterogeneity diversity during development. Microglia are highly heterogeneous in embryonic and early postnatal phases where several novel subpopulations of microglia were identified. There is limited transcriptional heterogeneity of microglia across anatomically distinct CNS regions in adulthood. Besides, the heterogeneity of microglia is relatively increased due to aging. Enriched genes significant to each microglial developmental stage revealed by recent studies are shown.

2.2 Spatial heterogeneity of microglia

The crosstalk mechanisms of distinct neuronal types and the differences in the neighboring astrocytes and stem cells may result in the regional specialization of microglia (Bohlen et al., 2017; Hammond et al., 2019; Kierdorf & Prinz, 2017). Recent studies at the single-cell level have provided a new sight into the spatial heterogeneity of microglia.

A study by Grabert et al. (2016) provided the first evidence that their regional localization influenced the genome-wide expression profile of microglia in adulthood. Specifically, they revealed the downregulation of differential genes (for example, triggering receptor expressed on myeloid cells 2 [*Trem2*], *Cd33*) involved in bioenergetic and immunoregulatory pathways. The upregulation of some other genes (for example, *Trem1*, *Cd300lb*) in cerebellar microglia compared with that in striatal and cortical microglia in the adult brain. A recent study with single-cell analysis has revealed the signatures of human microglial (huMG) heterogeneity (Böttcher et al., 2019). The authors identified the expression levels of 57 markers in huMG isolated from nine donors' five distinct brain regions (the subventricular zone, thalamus, cerebellum, temporal lobe, and frontal lobe). They confirmed that huMG has multiple phenotypic characteristics that seem closely related to the brain region in which they are located.

Moreover, the authors revealed comparatively higher expression of key markers related to microglial activation, such as CD68, CD86, and CX3CR1, in the subventricular zone (SVZ) and thalamus compared with that in other brain regions. Masuda et al. (2019) uncovered 10 microglial clusters with different transcriptional profiles by unbiased clustering and observed that the enrichment of cluster ten ($CST3^{+}SPARC^{+}IBA1^{+}$) lies above the cortex and hippocampus in juvenile mice, and cluster seven ($CST3^{-}SPARC^{-}IBA1^{+}$) was more prevalent in the adult cerebellum and corpus callosum. This study may be the first to present in vivo results comparing microglial heterogeneity at the single-cell level. Another study in mice highlighted that the transcriptomes of microglia in the ventral tegmental area are different from those in the cortex, substantia nigra pars reticulata, and nucleus accumbens, specifically concerning the genes involved in glycolysis and gluconeogenesis, as well as mitochondrial function and oxidative

phosphorylation (De Biase et al., 2017). In their recent transcriptome analysis, Lopes et al. (2022) identified the maximum number of differentially expressed genes between the SVZ and the two cortical regions, including the medial frontal gyrus and superior temporal gyrus. They observed that the expression levels of some genes (for example, *P2RY12*, *CD36*, *RC1*) were specifically upregulated. In contrast, those of others (for example, *FCER1A*, *IL15*, *RGS1*) were downregulated across the cortex, compared with that in the subcortical brain. They also found that the expression levels of some genes (for example, *CX3CL1*, *CCR2*, *FCGR3B*) decreased. In contrast, those of others (for example, *IL10*, *CLU*, *CD83*) considerably increased in the SVZ compared with that in the two cortical regions and thalamus.

Additional evidence has revealed that the progression of aging may impact regional microglial heterogeneity. Grabert et al. (2016) demonstrated the modest downregulation in the expression of key signature genes across all forebrain regions and the more significant effect in cerebellar microglia homeostasis-related genes (for example, Tmem119, P2ry12, P2ry13) resulting from aging, which maybe correlates with the degree of neuronal cell loss (Sobue et al., 2021), suggesting that the state of immune alertness of cerebellar microglia is further enhanced with aging. Soreq et al. (2017) corroborated with this and proposed that regional identity genes of glia can provide a more accurate human aging prediction than neuron-specific genes. A recent study also showed that most microglial phenotypes are generally consistent in the brain regions, with age–region relationships observed in only 91 genes when they subjected the genes to an interaction term model (Lopes et al., 2022). Among them, 35 genes (including *MRC1* and *CD24*) showed specific changes in the SVZ.

To date, other studies have yielded conflicting results that failed to detect the regional heterogeneity of cerebellum microglia by scRNA-seq analyses (Hammond et al., 2019; Li et al., 2019). Further research will be needed to resolve this controversy fully.

2.3 Sex and microglia diversity

Growing evidence suggests that sex can affect microglia's gene expression profile and physiological function throughout their lifespan (Guneykaya et al., 2018). Sex differences affect microglia's density, size, and phagocytic capacity (Hanamsagar et al., 2017; Schwarz et al., 2012). Temporal sex-related microglia heterogeneity has been revealed by analyzing transcripts of single microglia from males and females at different developmental stages, which indicated minor differences between sexes at the early postnatal time, with $CD74^{+}Ccl24^{+}Arg1^{+}$ microglia more frequently expressed in female brains (Hammond et al., 2019; Lopes et al., 2022).

Moreover, sex-related differences were more significant in the adult mice. A scRNA-seq study found that microglia in males displayed enrichment of genes related to inflammation and antigen presentation, and these microglia were more reactive to the stimulation of ATP (Guneykaya et al., 2018) and had a higher neuroprotective capacity than female microglia (Villa et al., 2018). These data show little sex-dependent variability in the microglia during development and homeostasis. More research on human or disease-associated microglia is required to uncover the effect of sex on microglial heterogeneity.

3. Microglia heterogeneity in a diseased brain

3.1 Microglia diversity involved in AD

AD is a detrimental neurodegenerative disorder, and microglia have been implicated in its pathogenesis. Keren-Shaul et al. identified a novel microglial subtype pertaining to neurodegenerative diseases, termed disease-associated microglia (DAM), placed by Keren-Shaul et al. through scRNA-seq (Keren-Shaul et al., 2017). DAM showed a distinct gene signature with high expression levels of *Cst7, Itgax*, *Apoe*, and *Lpl*. Histological analysis of mouse and human brain slices showed the localization of DAM to the area surrounding Aβ plaques. Single-cell and clustering analyses suggested that DAM is produced by a two-step mechanism of homeostatic microglial activation. DAM activation must be initiated in a TREM2-independent pathway, followed by activation of the TREM2-dependent program, suggesting complex mechanisms facilitating microglial heterogeneity. TREM2 has been reported to be an immune receptor expressed on microglia and a potential therapeutic target for stopping or delaying AD progression (Hickman & Khoury, 2014; Jeong et al., 2010; Jonsson et al., 2013).

Consistently, a recent study complemented previous work on the subject, confirming TREM2-dependent DAM at early and late stages of Aβ accumulation in 5XFAD mice via single-nucleus RNA sequencing (snRNA-seq) (Zhou et al., 2020). Rare loss-of-function variants in the gene encoding TREM2 have been reported to increase the risk of AD in humans (Ulland et al., 2018). Zhou et al. (2020) also revealed that compared with that in common variant (CV) samples, the expression level of non-homeostatic microglial genes IRF8, HLA-DRA, and allograft inflammatory factor 1 (AIF1) was significantly decreased in TREM2-R47H and TREM2-R62H samples. These results suggest there were fewer microglia in the R47H than in the CV TREM2 brain specimens and/or reduced expression of these microglial genes on a per-cell basis (Zhou et al., 2020).

Additional evidence of an AD-associated microglial phenotype has been identified by Olah et al. (2020). They segregated a new dataset based on 16,096 individual microglial transcriptomes into nine clusters that express microglia-enriched genes, such as *C1QA*, *C1QB*, *C1QC*. They found differences between two-component pathologies of AD (amyloid or tau), in which different microglial clusters were enriched, suggesting that different subtypes of microglia may be related to various aspects of AD. Moreover, a recent study found that significant differences in AD risk genes expressed in microglia (*APOE, MS4A6A, PILRA*) were related to tissue amyloid or pTau expression (Smith et al., 2022).

Reports on scRNA-seq technology in AD are increasing at an extraordinary rate. However, these studies have certain limitations owing to the difficulty in obtaining fresh autopsy samples and isolating live microglia from human brain tissue.

3.2 Microglia diversity involved in Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with a prevalence of about 1% in people over 65 (Sheng et al., 1998). With age, microglial phenotypic changes over time compromise their ability to maintain neuronal homeostasis, which may contribute to irreversible PD progression.

Agarwal et al. (2020) performed one of the first snRNA-seq analyses of human cortical and nigral tissues. They created an atlas based on healthy control tissues at the single-cell level. An enrichment of PD risk variants was found in neurons, and no disease association was identified in microglia. However, only control brain samples may conceal the association between PD and specific cell types. This opinion was confirmed in another recent study (Smajić et al., 2021), in which, using snRNA-seq, the authors identified a disease-specific upregulation of microglia in posthumous midbrain tissues from idiopathic PD patients and healthy people. In patients with PD, activated microglia split into two activation branches: one with high expression of GPNMB and the other with increased expression of IL-1β. *LRRK2* was highly correlated with PD in microglia, suggesting a significant enrichment of PD risk variants in microglia and neurons.

Limited by the availability of post-mortem brain tissue from patients with PD, human data published on this have been scarce. Previous studies have captured the complex regional heterogeneity of human microglia in PD patients (Lopes et al., 2022; Mastroeni et al., 2018; Zhong et al., 2021). Based on these studies, the pathways involved in neurodegeneration-related and PD risks and PD-specific activity, including inflammation-related aldosterone and reactive oxygen species metabolism, have been investigated at deeper levels in human microglia.

Current immunotherapy studies have focused on the progressive aggregation of α-synuclein in Parkinson's disease brain, targeting microglia for its ability to degrade extracellular α-syn (George et al., 2015). CB2 receptor on the microglia surface is also consideredan effective therapeutic target. In PD animal models, the administration of JWH133 (selective CB2 receptor agonist) inhibits the levels of reactive oxygen species and the production of pro-inflammatory factors (Chung et al., 2016). Moreover, The JAK/STA T and NF-κB pathways are the potential therapeutic targets (Hu et al., 2009). In animal PD models, administration of Tanshinone-I and α-asarone, selective inhibitors of the NF-κB pathway, can inhibit the differentiation of the classically activated (M1) microglia and reduce the levels of pro-inflammatory factors (Kim et al., 2015; Wang et al., 2015). Finally, activation of histamine receptor 4 (H4R) and the

NLRP3 inflammasome promotes the secretion of pro-inflammatory factors by microglia. In animal PD models, injection of an antagonist of H4R and NLRP3 can inhibit the activation of microglia, reduce damage to dopaminergic neurons, and improve animal behavior (Zhou et al., 2019). These studies show that several molecular targets can regulate the activation of microglia, but the results are still controversial (Brakedal et al., 2017; Connolly et al., 2015). Thus, an important microglial transcriptome should be further explored to provide insights into novel targets for immunotherapies.

3.3 Microglia diversity involved in neuroinflammation-related diseases

Neuroinflammation contributes to various brain pathologies, including infection, injury, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and lipopolysaccharide (LPS) challenge (Piancone et al., 2021).

MS, a neuroinflammatory autoimmune disease, is characterized by CNS demyelination. High similarity in cell state between demyelinated DAM and injury-responsive microglia has been reported, wherein this state induced the upregulation of similar distinct disease-associated genes (for example, *Apoe*, *Spp1*, and *Lpl*) (Hammond et al., 2019). As mentioned above, the expression of these genes is also upregulated in microglia surrounding plaques in AD models (Keren-Shaul et al., 2017). Masuda et al. (2019) isolated 422 $CD45^+$ cells from the brains of patients with early active MS using scRNA-seq and recognized one MS-associated and two MS-enriched clusters of microglia. They observed the downregulation of microglia core genes and increased expression levels of *APOE* and *MAFB*. The characteristics of MS-associated microglia are a high expression of *CSTD*, *APOC1*, *GPNMB*, *ANXA2*, and *LGALS1*. The expression of MHC class II-related molecules, such as *CD74*, *HLA-DRA*, *HLA-DRB1,* and *HLA-DPB1*, increased, and strong expression of *SPP1*, *PADI2*, and *LPL* transcripts were observed in the other MS-enriched clusters. Moreover, the proportion of subsets of microglia among different patients was significantly different, suggesting high inter-individual heterogeneity of microglia during the disease progression of MS.

ALS is a progressive neurodegenerative disorder that affects motor neurons. Liu et al. (2020) found that the transcriptome data for most cell types were altered in mice carrying mutated superoxide dismutase 1 (*SOD1*)—a mouse model of familial ALS. Their results suggested that the specific cell types with perturbations in known ALS pathways are not limited to motor neurons. Thus, these results suggest novel targets for investigating the pathogenic mechanisms of neuroinflammation-related diseases.

Peripheral administration of LPS produces pro-inflammatory cytokines by activating microglia and is frequently used to mimic neuroinflammation-associated diseases (Jeong et al., 2010; Zhao et al., 2019). Sousa et al. (2018) compared the inflammatory-associated microglia (IAM) signature with DAM and reported that Trem2 and Tyrobp expression levels were highly decreased in IAM; however, upregulation of both genes has been reported in DAM. In addition, the expression of genes related to CNS diseases, such as *Cd33*, *Cd9*, and *Sod1*, was downregulated in IAM compared with that in DAM. These results indicate the heterogeneity of microglial responses to systemic inflammation.

3.4 Glioma and microglia heterogeneity

Considerable progress has been made in uncovering the transcriptomic heterogeneity of glioma-associated microglia (GAM), allowing for further analysis of microglial heterogeneity in glioma and other brain malignancies (Gosselin et al., 2017). The researchers conducted the first scRNA-seq study of myeloid cells infiltrating human brain tumors in isocitrate dehydrogenase-mutant adult glioblastomas (Venteicher et al., 2017). They demonstrated a phenotypic spectrum ranging from microglia to macrophage-like cells, according to the gradual variation in the expression of microglia and macrophage markers. A recent study revealed human microglia heterogeneity and the disease-associated phenotype by profiling microglia from glioblastomas using scRNA-Seq and mass cytometry (CyTOF) (Sankowski et al., 2019). Nine microglial clusters were identified in this study. GAM clusters downregulated microglia core genes, including *CX3CR1* and *SELPLG,* and upregulated metabolic, inflammatory, and interferon-related genes, such as interferon-induced protein 44 (*IFI44*), *SPP1*, *HLA-DRA*,

APOE, and *CD163*. In addition, GAMs exhibit context-dependent upregulation of other genes, such as *CD163* and *VEGFA* (Sankowski et al., 2019).

Interestingly, microglia tended to be mainly located at the edge of the tumor and adjacent brain parenchyma (Darmanis et al., 2017; Friebel et al., 2020; Ochocka et al., 2021). The expression of genes encoding cytokines (*CCL3*, *CCL4*, *TNF*) and pro-inflammatory interleukins (*IL1B*, *IL1A*, *IL6R*) increased in myeloid cells isolated from the peripheral tumor zones. In contrast, the expression of *VEGFA*, *VEGFB*, and *IL1RN* was upregulated in immune cells isolated from the core tumor zones, revealing the tumor-supportive phenotype of GAMs within the tumor core zone (Darmanis et al., 2017). Taken together, the context-dependent microglial phenotype in the brain remains uninvestigated, thereby warranting further studies for identifying novel therapeutic targets in unique GAM subpopulations.

3.5 Microglia in major depressive disorder

Major depressive disorder (MDD) is a common psychiatric disorder. Several studies have revealed that microglia may be involved in the progression of depression disorder by producing pro-inflammatory cytokines and their metabolic products, which is also consistent with our previous work (Jia et al., 2021; Wang et al., 2018; Wang et al., 2021). In contrast, a scRNA-seq study of microglia from four mouse brain regions (cortex, cerebellum, hippocampus, striatum) first demonstrated a non-inflammatory phenotype of microglia in MDD (Böttcher et al., 2020). This study was conducted by the same research group as mentioned above (Masuda et al., 2019); they reported decreased levels of HLA-DR and CD68, but comparable levels of inflammation-associated molecules (for example, IL-1β, IL-6, TNF, CCL4, IL-10, and CCL2) in microglia from patients with MDD. Increased levels of the homeostatic proteins P2Y12 receptor, TMEM119, and CCR5 (CD195) have been found in microglia from all brain regions of patients with MDD (Böttcher et al., 2020). These findings indicate augmented homeostatic functions of microglia, and complement the concept of neuroinflammation in mood disorders in MDD.

4. Conclusion

Reports on single-cell technologies are growing astonishingly, providing new insights into the regional heterogeneity and functional diversity of microglia, thus significantly extending our understanding of neurological disorders. Nevertheless, issues, such as technology and sample acquisition, have not yet been fully addressed.

Confirming genes relevant to the disease still requires a lot of work. Researchers should apply scRNA-seq to a particular disease to define a gene expression profile at the single-cell level. Although single-cell technology can identify specific microglia subtypes at different developmental and pathological states, their roles and interactions still require further exhaustive studies. Owing to the correlation between diseases and many secondary pathologies, cellular heterogeneity at the protein level should also be considered to determine the interaction between cell phenotype and functional state. Another limitation is the difficulty of obtaining freshly collected autopsy samples. In studies of human brain diseases, the brain tissues of the control group are rarely from healthy samples but nonpathological tissues in epilepsy samples.

Technically, results from scRNA-seq or CyTOF analyses, which help analyze a limited number of target molecules, only describe a subset of microglia molecular phenotypes. Furthermore, the combination of both approaches is advantageous (Sankowski et al., 2019; See et al., 2017); however, they have been investigated in different cells, hindering accurate multi-omics measurements of individual cells. Simultaneous measurements of single-cell mRNA and protein expression through oligonucleotide-labeled antibodies during CITE-Seq measurements can compensate for these single-cell methods (Stoeckius et al., 2017). Moreover, another novel approach for single-cell analysis, such as single-cell ATAC-sequencing or single-cell protein analysis, such as REAP-seq, will aid in a comprehensive analysis.

In the future, novel techniques will help us address many unanswered questions and open new avenues for developing therapeutic targeting of microglia.

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Conflict of Interest Disclosures

The authors declare that they have no conflict of interest.

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Abbreviations

APOE	Apolipoprotein E
CAMs	CNS-associated macrophages
CNS	Central nervous system
CX3CR1	CX3C chemokine receptor 1
DAM	Disease-associated microglia
GAM	Glioma-associated microglia
GPNMB	Glycoprotein non-metastatic melanoma protein B
huMG	Human microglia
IAM	Inflammatory-associated microglia
LPL	Lipoproteinlipase
LPS	Lipopolysaccharide
MDD	Major depressive disorder
MS	Multiple sclerosis
MS4A	Membrane-spanning 4-domains A
P2RY12	P2Y12 purinoceptor
PAM	Proliferative-region-associated microglia
PD	Parkinson's disease
ScRNA-seq	Single-cell RNA sequencing
SnRNA-seq	Single-nucleus RNA sequencing
SOD1	Superoxide dismutase 1
SPP1	Secreted phosphoprotein 1
SVZ	Subventricular zone
TMEM119	Transmembrane protein 119
TREM2	Triggering receptor expressed on myeloid cells 2