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REVIEW

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Single-cell Transcriptional Changes in Neurodegenerative Diseases

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Abstract—In recent decades, our understanding of the molecular changes involved in neurodegenerative diseases has been transformed. Single-cell RNA sequencing and single-nucleus RNA sequencing technologies have been applied to provide cellular and molecular details of the brain at the single-cell level. This has expanded our knowledge of the central nervous system and provided insights into the molecular vulnerability of brain cell types and underlying mechanisms in neurodegenerative diseases. In this review, we highlight the recent advances and findings related to neurodegenerative diseases using these cutting-edge technologies.

Key words: single-cell RNA sequencing, neurodegenerative disease, Alzheimer's, Parkinson, Huntington, multiple sclerosis.

INTRODUCTION

Neurodegenerative diseases are chronic and progressive illnesses related to the central nervous system (CNS), identified by the loss of neurons in the brain (Costa et al., 2013). Many efforts at the molecular level have tried to understand the basic biological mechanisms contributing to neurodegeneration (Wang et al., 2009; Casamassimi et al., 2017). Although the main focus has been placed on the role of protein metabolism and aggregates in neurodegenerative diseases (NDDs), researchers try to decipher the role of transcriptomic alteration as a contributing factor in the pathogenesis of these diseases (Suntsova et al., 2019).

RNA-SEQUENCING TECHNIQUES

Next-generation RNA sequencing (RNA-Seq) has become increasingly common for high-throughput transcriptome analysis and revolutionized our understanding of the molecular etiology of human disease (Wang et al., 2009; Costa et al., 2013; Casamassimi et al., 2017). This technology reveals both the presence and quantity of any transcript in multiple human tissues affected by different disorders (Costa et al., 2013; Suntsova et al., 2019). However, the conventional RNA-Seq technique usually needs micrograms of total RNA, which corresponds to hundreds of thousands of cells. Getting such a large amount of RNA from biological samples with a limited number of cells can be practically impossible (Tang et al., 2009). Besides, every cell subpopulation in a given tissue may express a unique transcriptome (Huang, 2009). Thus, bulk population sequencing measures the average expression of transcripts and likely results in missing essential data about cell-to-cell variability of gene expression (Hwang et al., 2018). To overcome these challenges, Tang et al. (2009) introduced a single-cell RNA sequencing approach (scRNA-Seq) to study a single mouse blastomere (Fig. 1). Their results demonstrated the advantage of scRNA-Seq over single-cell microarray in detecting the expression of more genes and new splice variants at a single-cell resolution. Since this first report in the field (Tang et al., 2009), several scRNA-Seq platforms have been developed (Fig. 2). They fall into two categories: Droplet-based (e.g., Drop-Seq, inDrop, Seq-well, 10x Chromium Genomics) and plate-based (e.g., MARS-Seq, Smart-Seq,

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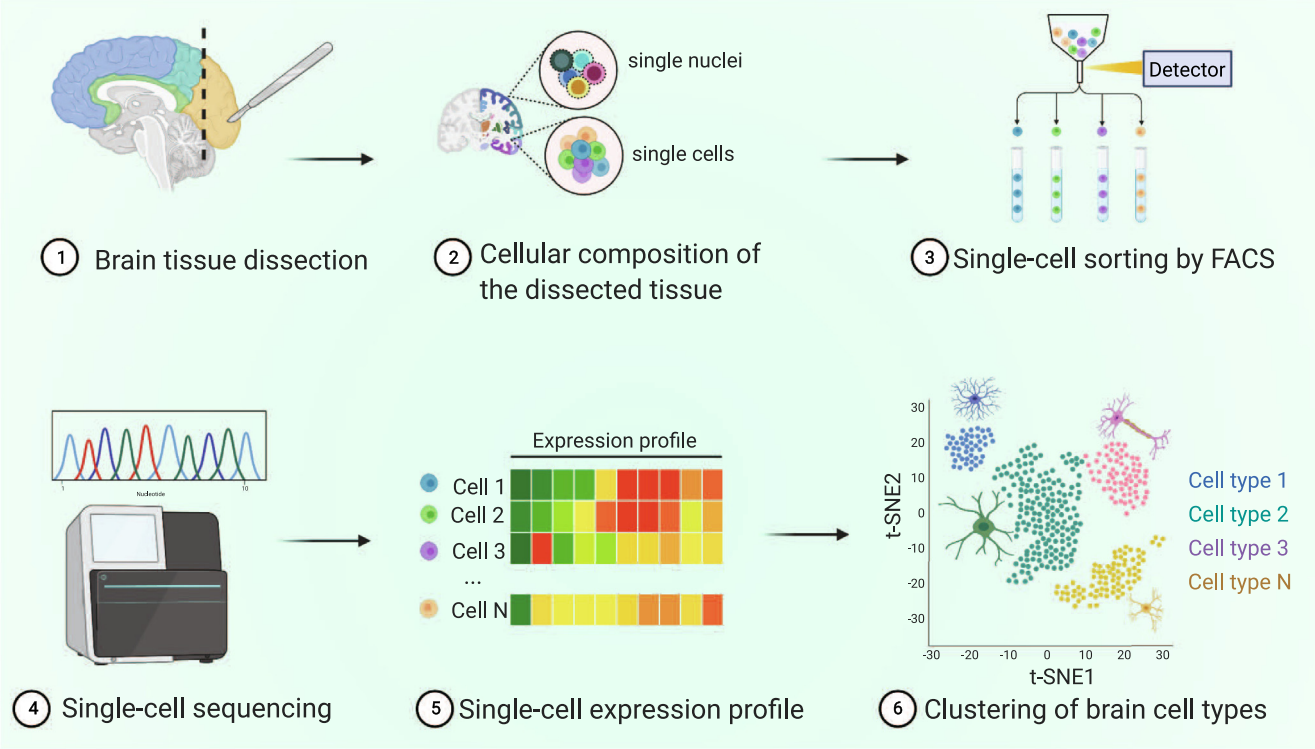

Fig. 1. Single-cell technologies. Single-cell and single-nucleus RNA sequencing has provided unique insight into the transcriptomic changes that occur in the brain.

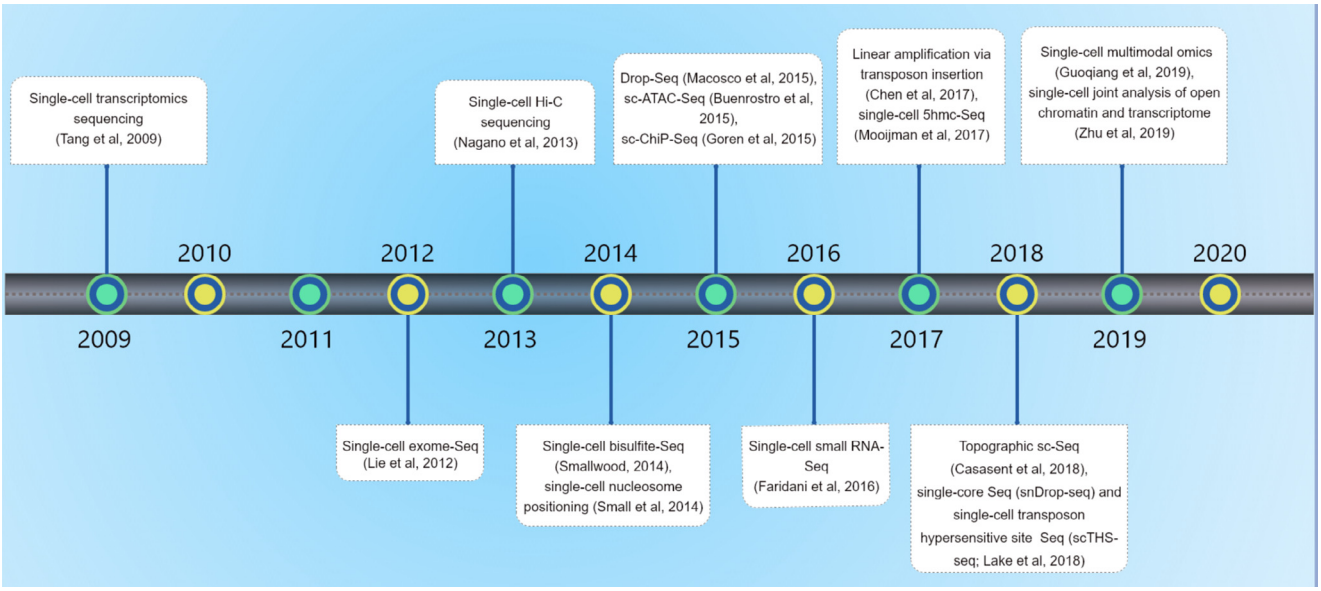

Fig. 2. Timeline of single-cell sequencing methods milestones.

Smart-Seq2, and STRT-Seq) based on their downstream methods (Szczelkun et al., 2019, 2019). Recently, single nuclei RNA sequencing (snRNA-Seq) has also been introduced, which affords some advantages over scRNA-Seq including comparable gene detection to scRNA-seq, reduced dissociation bias, and compatibility with frozen samples. In contrast, scRNA-seq methods are most appropriate in situations when cells cannot be harvested intact and viable, which is often true for preserved tissues, and always true for some cell types (e.g. neurons, adipocytes) (Bakken et al., 2018). All scRNA or snRNA-Seq platforms share four steps: isolation of single cells or nuclei, reverse transcription, cDNA synthesis, and sequencing; but may utilize different techniques in each step (Hedlund and Deng, 2018). The advantages and drawbacks of each technique are comprehensively

reviewed elsewhere (Hu et al., 2016; Nguyen et al., 2018; Ding et al., 2019; Baran-Gale et al., 2018, 2018). These techniques are currently increasingly used to identify, characterize, and classify cell subpopulations in healthy and diseased states of the brain (Cuevas-Diaz Duran et al., 2017). In this regard, a few single-cell databases have also been created to facilitate access to the most updated findings using single-cell technologies (Table 1).

As our understanding of cell populations susceptible to neurodegenerative diseases is of high importance (Jiang et al., 2020), the present review will further focus on the transcriptional changes in neurodegenerative diseases at the single-cell resolution.

APPLICATION OF SCRNA-SEQ FOR NEURODEGENERATIVE DISEASES

A major challenge in understanding the pathogenesis of neurodegenerative diseases and brain aging is to determine the body's intrinsic mechanisms that could be causative or protective against neurodegeneration. For instance, increasing reports suggest that disease-associated microglia (DAM), a recently identified subset of microglia found at the damaged regions, might have a protective role. This subpopulation was first identified in a mouse model of Alzheimer's disease by single-cell RNA-seq (Keren-Shaul et al., 2017). DAMs are molecularly identified with expressing microglial markers, *Iba1*, *Cst3*, and *Hexb*, and downregulation of homeostatic microglial genes, including *P2ry12*, *P2ry13*, *Cx3cr1*, *CD33*, and *Tmem119* (Butovsky et al., 2014). DAMs also show upregulation of genes involved in lysosomal, phagocytic, and lipid metabolism pathways, including several known AD risk factors, such as *Apoe*, *Ctsd*, *Lpl*, *Tyrobp*, and *Trem2* (Lambert et al., 2013). This subset of cells has been proposed to have a dedicated sensory mechanism to detect damage within the damaged brain in the form of neurodegeneration-associated molecular patterns (NAMPs), which has been fully discussed elsewhere (Deczkowska et al., 2018). Here we discuss the involvement of different cell types in neurodegeneration in more detail.

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common cause of dementia, which starts with mild memory loss and eventually impairs executive and cognitive functions (Tarawneh and Holtzman, 2012; Kirova et al., 2015; Ahmadi et al., 2020). Extracellular deposition of amyloid plaques and intracellular accumulation of hyperphosphorylated tau proteins are the pathological hallmarks of AD (Fig. 3) (Perl, 2010). AD is usually defined by different stages: mild cognitive impairment (MCI) in which people have mild changes in their memory and thinking ability; mild dementia when having significant trouble with memory and thinking that impacts daily functioning; moderate dementia when people grow more confused and forgetful and begin to need more help with daily activities and self-care; and severe dementia when mental function continues to decline, and the disease has a growing impact on movement and physical capabilities (Perl, 2010). Hippocampus, entorhinal cortex, and the medial temporal lobe are brain regions known to be affected early during the disease process, whereas the sensory cortex and motor cortex are known to be rather spared (Xu et al., 2019). Different types of cells such as glia, neurons and vascular cells (Sweeney et al., 2018) are affected by AD in these regions (Miller et al., 2013; Narasimhan et al., 2020). Several gene expression profiling studies have been performed over the past two decades to understand the molecular complexity that drives AD pathogenesis (Loring et al., 2001; Blalock et al., 2004; Liang et al., 2008; Liang et al., 2008; Berchtold et al., 2013; Miller et al., 2013; Magistri et al., 2015; Wang et al., 2016; Hokama et al., 2014, 2014). However, the precise transcriptional changes across different cells, particularly minor cell populations in an AD brain, cannot be practically obtained by profiling studies on bulk samples (Mathys et al., 2019). Mathys et al. (2019) utilized an snRNA-Seq approach to find gene expression changes in the prefrontal cortex region of patients with varying AD pathology degrees. Analyzing single nucleus transcriptomes showed that all major cell types of the prefrontal cortex region were affected by AD pathology at the transcriptional level. However, in excitatory and inhibi-

Table 1. A list of available single-cell-specific databases

Database	Data type	Species	Accession/URL	Ref.
scRNASeqDB	transcriptomics	Human	https://bioinfo.uth.edu/scrnaseqdb/	(Cao et al., 2017)
PanglaoDB	transcriptomics	Human/mouse	https://panglaodb.se/	(Franzén et al., 2019, 2019)
Single-cell portal	Genomics, transcriptomics, proteomics	Human/mouse/cell lines and other animal models	https://singlecell.broadinstitute.org/single_cell	
EMBL-EBI single-cell expression atlas	transcriptomics	Animals/plants/fungi/protists	https://www.ebi.ac.uk/gxa/sc/home	
Allain brain map	transcriptomics	Human/mouse	https://portal.brain-map.org/atlases-and-data/rnaseq	
SCDevDB	transcriptomics	Human	https://scdevdb.deepomics.org/	(Wang et al., 2019)
scREAD	transcriptomics	Human/mouse (Alzheimer's)	https://bmbls.bmi.osumc.edu/scread/	(Jiang et al., 2020)

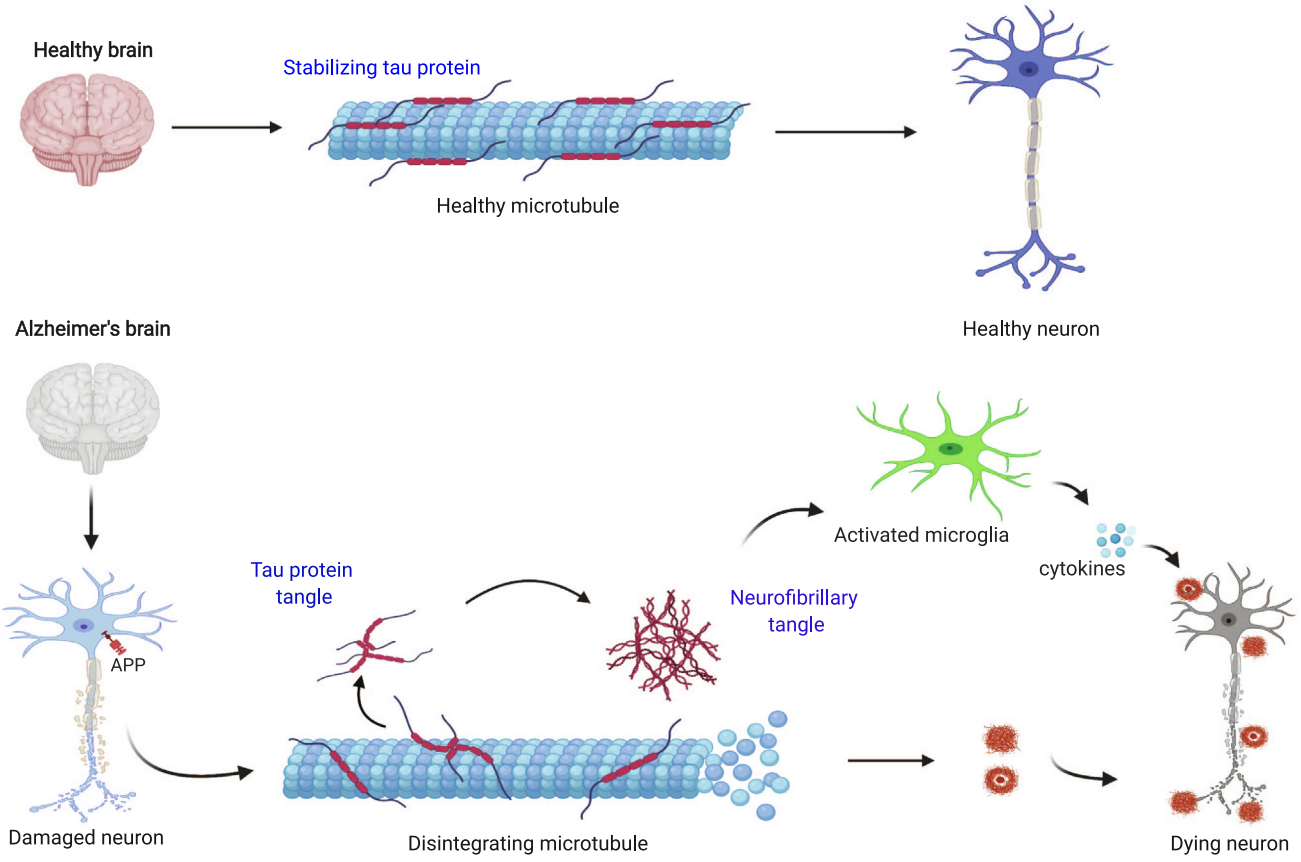

Fig. 3. Pathobiology of Alzheimer's diseases. The illustration depicts the pathological properties of the AD brain (involving tau protein) with that of the healthy brain.

tory neurons, most differentially expressed genes (DEGs) were downregulated, whereas most DEGs in astrocytes, microglia, and oligodendrocytes presented an upregulation trend (Mathys et al., 2019).

Likewise, an analysis of DEGs using bulk RNA-Seq data revealed predominant expressional changes in excitatory neurons and oligodendrocytes (Mathys et al., 2019). However, bulk RNA-Seq could not detect DEGs like *APOE* with opposite directionality across all cell types, which was down- and upregulated in astrocytes and microglia, respectively. The majority of DEGs were cell-type specific with top DEGs including *LINGO1*, *ERBIN*, *CNTNAP2*, *NEGR1*, *BEX1*, *NTNG1* involved in processes such as myelination, axonal outgrowth, and regeneration across different cell types. These processes were suggested to be affected as a regulatory response to maintain myelin integrity during AD pathogenesis. Comparing gene expression profiles between early pathology (amyloid burden, but modest neurofibrillary tangles and modest cognitive impairment) and late pathology (higher amyloid burden, and also increased neurofibrillary tangles, global pathology, and cognitive impairment) subgroups revealed that significant transcriptional changes appeared early in pathological progression and were mostly cell type-specific (Mathys et al., 2019). Besides, although upregulated genes were common across cell types in patients with late-stage pathology and mainly encoded proteins involved in maintaining protein integrity, the majority of the downregulated genes were cell-type specific. Cell types such as excitatory neurons, inhibitory neurons, astrocytes, microglia, and oligodendrocytes each represented distinct correlations with multiple pathological traits, indicating different groups of genes respond to AD pathology in each cell type (Mathys et al., 2019).

Quantifying overrepresentation of genes from genome-wide association studies (GWAS) revealed a link between transcriptional responses to AD pathology and genetic risk factors for AD (such as *APOE*, *TREM2*, *MEF2C*, *PICALM*) in both neurons and glial cells (Mathys et al., 2019). Also, different sets of cell subpopulations were associated with different AD-pathology traits (*i.e.*, neuronal neurofibrillary tangle density, neurofibrillary tangle burden, global AD-pathology burden, the global measure of neocortical pathology, neuritic plaque burden, overall amyloid level, and global cognitive function) and the expression pattern of the genes specific to each subpopulation. Also, sex-specific transcriptional responses to AD-pathology were observed in multiple cell types particularly in neurons and oligodendrocytes (Mathys et al., 2019). For example, a global transcriptional activation in oligodendrocytes was correlated with increased pathology in males, whereas in females, increased pathology was correlated with a global downregulation of gene activity in both excitatory and inhibitory neurons. The reduced

transcriptional response, particularly in oligodendrocytes, was suggested to be linked to white-matter changes in females with AD pathology (Gallart-Palau et al., 2016).

As mentioned above, investigating molecular changes in the damaged brain of mouse models identified the role of some cell types such as DAMs in AD (Keren-Shaul et al., 2017). Single-cell analysis of DAM in transgenic AD mouse models revealed that the DAM program is activated in a two-step process, initially in a Trem2-independent manner that involves downregulation of microglia checkpoints, followed by activation of a Trem2-dependent program. In 5XFAD and APP/PS1 models of AD, DAM co-localized with Aβ plaques in the cortex and were absent from the cerebellum, where Aβ plaques do not occur (Mrdjen et al., 2018).

To characterize the cellular heterogeneity and transcriptional changes in the entorhinal cortex region of AD patients' brains, Grubman et al. (2019) analyzed 13,214 cells using snRNA-Seq. The DEGs that overlap between this study and the Mathys et al. study (Mathys et al., 2019) showed high concordance (> 90%) of effect across the major cell types. Their results revealed both cell-independent and cell-type-specific transcriptional alterations in the human AD brain's entorhinal cortex. Genes involved in response to misfolded proteins and cell stress were coordinately regulated across multiple cell types, possibly as a response to extracellular amyloid deposition (Su et al., 2020). Also, astrocytes, endothelial cells, and microglia were the cell types with the most coordinated gene expression differences between AD patients and healthy individuals. Upregulated genes in endothelial cells were involved in cytokine secretion, immune responses, and processes related to neurodegeneration, while downregulated genes in neurons, oligodendrocytes, astrocytes, and oligodendrocyte precursor cells (OPCs) were related to behavior, cognition, and synapse organization functions. In contrast, upregulated genes in neurons, astrocytes, and oligodendrocytes were related to glial cell development, differentiation, and myelination. Furthermore, genes involved in homeostasis, cell–cell adhesion, lipid response, and G-protein-coupled receptor pathways were downregulated in AD microglia. Besides, genes related to synaptic transmission and ion transport and mechanisms involved in memory were downregulated in excitatory and inhibitory neurons, respectively (Grubman et al., 2019).

AD-associated transcriptional changes were also observed in most subclusters of each cell type. The differential expressions were particularly evident for CNS-related genes like *LINGO1*, *NEAT1*, and *GRID1*. In addition, the molecular identity of specific neuronal subsets (possibly as deep layer neurons) was more susceptible to AD. Additional analysis of the expression of 1000 GWAS candidate genes displayed cell-type-specific expression patterns. For instance, *APOE* revealed downregulation in a subcluster of OPC, oligodendrocyte, and two subclusters of astrocytes, and upregulation in a subcluster of microglia. The relationship of regulatory factors such as the TFEB transcription factor and AD-associated GWAS loci in astrocytes showed subcluster-specific regulation of AD genes (Grubman et al., 2019). These data demonstrated a functional link between AD and certain astrocyte subpopulations.

AD-related single-cell transcriptional changes have also been observed in other human brain areas including the caudal entorhinal cortex (affected early in the course of AD) and the superior frontal gyrus (affected late in AD) of patients carrying the *APOE* ε3/ε3 genotype (Leng et al., 2021). Although there was a downward trend in the relative abundance of excitatory neurons in Braak stages II and VI in the entorhinal cortex and Braak stage VI in the superior frontal gyrus, no differences were observed in the vulnerability of inhibitory neurons. Three subpopulations of entorhinal cortex excitatory neurons (EC: Exc.s2, EC: Exc.s4, and EC: Exc.s1) were defined as selectively vulnerable excitatory neurons, of which EC: Exc.s2 displayed the largest number of downregulated genes. These genes encode for pre-and postsynaptic proteins. Similar transcriptional patterns of two excitatory neuron subpopulations (Superior frontal gyrus (SFG): Exc.s2 and SFG: Exc.s4) in the SFG suggest similar selective vulnerability mechanisms in different brain regions in AD (Leng et al., 2021). This was confirmed by observing transcriptional similarity (*e.g.*, expression of *RORB* gene) between excitatory subpopulation 4 in the prefrontal cortex of male participants (Mathys et al., 2019; Marinaro et al., 2020) and entorhinal cortex (EC): Exc.s2 and EC: Exc.s4 cell types (Leng et al., 2021). Although the increased relative abundance of microglia was observed in the entorhinal cortex in AD progression, the expression of DAM markers was not detected (Leng et al., 2021). In contrast, Olah *et al*. observed a reduced frequency of a microglia cluster in the dorsolateral prefrontal cortex and the temporal cortex regions of AD patients, which was enriched for AD-related genes (Olah et al., 2020).

By sub-clustering of oligodendrocytes in the entorhinal cortex and superior frontal gyrus, Leng et al. (2021) revealed subpopulations (EC: Oligo.s0 and EC: Oligo. s4; SFG: Oligo.s1 and SFG: Oligo.s2) that exhibited higher expression of AD-associated oligodendrocyte genes similar to subpopulation Oli0 in a previous study (Mathys et al., 2019). Subclustering of astrocytes revealed that three subpopulations (EC: Astro.s3, SFG: Astro.s4, and SFG: Astro.s5) expressed dramatically higher levels of GFAP, a marker of reactive astrocytes, and were referred to as $GFAP^{high}$ astrocytes (Leng et al., 2021). These subpopulations behave identically compared to astrocyte subpopulation 2 of Mathys et al. study in upregulating reactive astrocyte markers and downregulating genes associated with glutamate/GABA homeostasis and synaptic adhesion (Leng et al., 2021).

Although both scRNA-Seq and snRNA-Seq technologies are becoming popular for transcriptional profiling of AD, recent evidence suggests that snRNA-Seq is not suitable for the detection of microglial activation genes in humans (Thrupp et al., 2020). Thrupp et al observed that a group of genes (∼1%) is depleted in nuclei compared to whole cells. This set of genes is enriched for microglial activation genes, including *APOE*, *CST3*, *SPP1*, and *CD74*, comprising 18% of previously

identified microglial-disease-associated genes (Thrupp et al., 2020). Altogether, snRNA-Seq results highlight the mechanisms of cell-type expressional heterogeneity and dysfunction in AD and may also explain why the whole-body gene knockouts in AD models have often yielded discrepant results (Götz et al., 2004; Leung and Jia, 2016).

PARKINSON'S DISEASE

Parkinson's disease (PD) is the most common neurodegenerative movement disorder that affects 2–3% of the population over 65 years of age (Poewe et al., 2017). Loss of dopaminergic (DA) neurons in the substantia nigra (SN) and intracellular aggregation of the α-synuclein protein are the main hallmarks of PD (Fig. 4) (Poewe et al., 2017; Balestrino and Schapira, 2020). However, the heterogeneity of DA neurons and the reason for their vulnerability to degeneration remains mainly unknown. Single-cell transcriptional analysis of forebrain, midbrain, and olfactory bulb regions of mice showed all DA subtypes, except those in SN and periaqueductal gray area, expressed either GABAergic or glutamatergic markers during development (Hook et al., 2018). Postnatal SN DA neurons revealed 110 marker genes significantly associated with PD, suggesting their role in preferential susceptibility of the SN in PD. Furthermore, 24 PD-related GWAS loci represented association with single genes including *Mmp16*, *Tsnax*, *Satb1*, *Snca*, *Pdzrn4*, and *Gch1* in SN DA neurons, revealed by scRNA-Seq (Hook et al., 2018).

In vitro studies (Lang et al., 2019) using bulk RNA-Seq on iPSC-derived DA neurons from PD patients, carrying the most common variant (GBA-N370S) in the glucocerebrosidase gene, revealed DEGs involved in neuronal development and differentiation, synaptic activity, and zinc ion transport (Lang et al., 2019). Instead, scRNA-Seq results initially stratified GBA-N370S carriers based on the expression of genes belonging to the SRP-dependent co-translational protein targeting the membrane pathway. Overall, 60 genes were consistently deregulated in PD GBA-N370S iPSC-derived DA neurons, using both techniques (Lang et al., 2019). Genes downregulated at early stages of the disease were implicated in neuronal function, microtubule function and formation, microtubule-associated protein tau splicing, neurite and axonal outgrowth, protein secretion and traf-

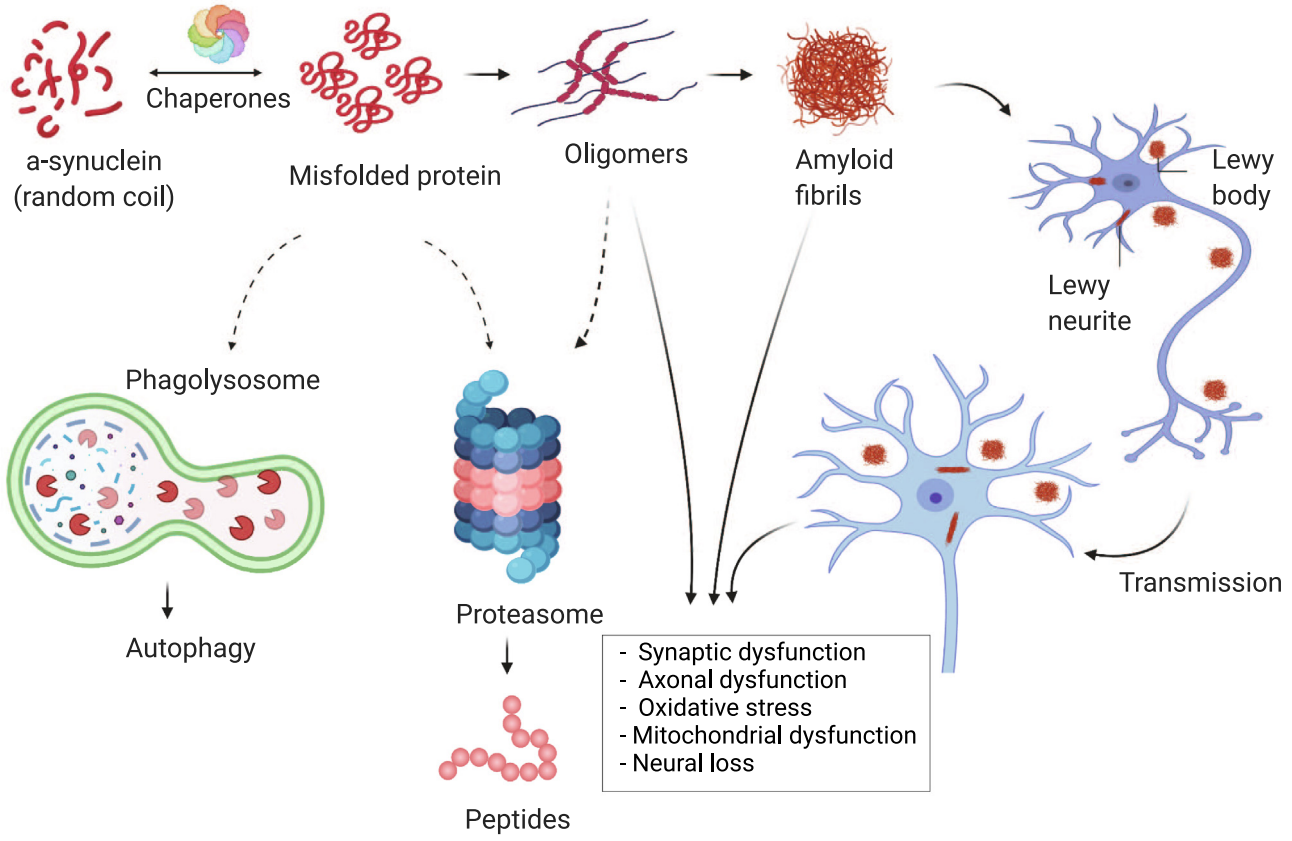

Fig. 4. A schematic of the pathology of PD. In normal situations, α-synuclein exists in a soluble random coil state. In pathological states, pathogenic variants of α-synuclein (dimers, trimers, and oligomers) are resulted due to misfolded α-synuclein that further aggregate into other toxic forms (such as amyloid fibrils) in Lewy bodies and Lewy neurites. Healthy quality control systems such as chaperones, ubiquitin proteasomes, and phagolysosomes that clear misfolded proteins are overwhelmed by oligomeric species of α-synuclein. α-synuclein fibrils are able to transmit disease (α-synucleinopathy) between neurons and cause clinical disease.

ficking, and protein kinase C pathway, whereas ER stress-related genes were upregulated in late phases. Importantly, HDAC4 mislocalized to the nucleus was found as a repressor of a set of the downregulated genes in the PD GBA-N370S iPSC-derived dopaminergic neurons. These results suggested that the modulators of HDAC4 activity or localization could correct PD-related cellular phenotypes (Lang et al., 2019).

Another similar *in vitro* experiment using iPSC-derived DA neurons displayed eight individual clusters consisting of two neuron progenitor populations and four dopaminergic neuron populations (DAn1–DAn4) (Fernandes et al., 2020). All dopaminergic neurons, particularly DAn1, were closely linked with substantia nigra mouse and human neurons reported by previous studies (Fernandes et al., 2020). Moreover, an *in vitro* model of PD generated via treating neurons with rotenone, a toxic pesticide causing PD-like symptoms, exhibited a significant reduction in the proportion of DAn1 with differences in synaptic transmission, lipid biosynthesis, and neuronal apoptotic genes and a strong representation of DAn2 neurons in the post-treatment culture (Fernandes et al., 2020). The opposing regulation of heat shock stress response and cell death genes in DAn1 and DAn2 neurons was found to be a factor underlying the selective vulnerability of these dopamine neurons in response to oxidative stress (Fernandes et al., 2020). The impact of a PD-associated genetic mutation (A53T) on the expression profile of dopamine neurons differentiated from SNCA-A53T mutant iPSC demonstrated activation of stress and cell death pathways in mutant DAn1 cells. Treatment of SNCA-A53T mutant dopaminergic neurons with rotenone -a pesticide that is used to generate animal models of PD (Huang et al., 2018)- led to robust upregulation of glycolysis and cholesterol biosynthesis genes in DAn1 mutant cells. Altogether, results demonstrate transcriptional changes exclusively in DAn1 neurons under cytotoxic and genetic stressors, suggesting a differential response of subtypes of dopamine neurons to different stressors (Fernandes et al., 2020).

Although most of the research in PD has focused on nigrostriatal DA neurons, the main findings still show the involvement of cell types such as astrocytes and microglia in PD pathogenesis (Brück et al., 2016; Booth et al., 2017; Reynolds et al., 2019). Integrative analysis of GWAS and scRNA-Seq data exhibited enrichment of autophagy genes in oligodendrocytes and cholinergic/mono-aminergic neurons, the lysosomal gene set in microglia, and the mitochondrial gene sets in almost all brain cell types (Reynolds et al., 2019). This suggests that PD risk loci lie in global cellular processes detectable across different cell types.

To understand the role of microglial in the pathobiology of PD, Huarte et al. (2021) combined single-cell RNA-sequencing with immunofluorescence analyses of the murine nigrostriatal region, the most affected brain region in PD. A microglia subpopulation, which is mainly present in the midbrain, showed a transcriptional signature related to immune pathways sharing features of inflammation-induced microglia. In addition, an *in situ* morphological screening of inferred cellular diversity displayed a reduced microglia complexity in the midbrain in comparison with the striatum. This suggests the involvement of specific microglia phenotypes within the nigrostriatal pathway related to PD pathogenesis (Uriarte Huarte et al., 2021).

In addition to *in vitro* and animal model studies, snRNA-seq analysis of post-mortem brain tissues from idiopathic PD (IPD) individuals (Smajic et al., 2020) identified PD risk variants associated with microglia and neuronal-specific genes, but less associated with OPCs and astrocytes-specific genes (Smajic et al., 2020). Cell-type compositional changes revealed an increase in the fraction of microglia and astrocytes and a decreased fraction of oligodendrocytes in IPD midbrains. Also, a negative association of male sex with the neuronal cells, inhibitory, excitatory, GABA, and dopaminergic neurons suggests that PD pathophysiology might affect the midbrain cellular composition in a sex-dependent manner (Smajic et al., 2020). PD-risk variants presented an association with glia- and neuron-specific gene expression patterns. In addition, microglia and astrocytes revealed IPD-specific cell proliferation and dysregulation of genes involved in unfolded protein response and cytokine signaling. IPD-microglia revealed a specific pro-inflammatory trajectory. A neuronal cell cluster exclusively present in IPD midbrains identified by *CADPS2* overexpression and a high proportion of cycling cells. This implies that increased expression of *CADPS2* is specific to dysfunctional dopaminergic neurons, which have lost their dopaminergic identity and unsuccessful attempt to re-enter the cell cycle (Smajic et al., 2020). Altogether, despite these few scRNA-Seq studies, more research needs to be performed, especially on different human brain regions, to improve our understanding of PD etiology at the level of different cell types.

HUNTINGTON'S DISEASE

Huntington's disease (HD) is an inherited neurodegenerative disease caused by a CAG trinucleotide repeat expansion in the *HTT* gene (Fig. 5) (Ross et al., 2014). Patients with HD show motor, cognitive, and psychiatric symptoms in midlife (Ghosh and Tabrizi, 2018). The major pathology site is basal ganglia, and projection neurons are the major cell types affected by HD in both striatum and globus pallidus subdivisions (Reiner et al., 2011). Cell loss and shrinkage were also detected in other brain areas including the cerebral cortex (Selemon et al., 2004), amygdala (Kipps et al., 2007, 2007), thalamus (Douaud et al., 2006), hypothalamus (Petersén et al., 2005, 2005), substantia nigra (Douaud et al., 2006), cerebellum (Vonsattel, 2007), and brainstem (Koeppen, 1989). Transcriptional analysis of blood and postmortem brain samples of HD patients using RNA-Seq (Hensman Moss et al., 2017) exhibited dysregulation of genes involved in the immune system (Neueder and Bates, 2014; Mastrokolias et al., 2015; Miller et al., 2016), alternative splicing (Lin et al., 2016), neuroinflammation, and development (Labadorf et al., 2015). However, due to the limitation of bulk RNA-Seq in attributing DEGs mainly to astrocytes and microglia (Hodges et al.,

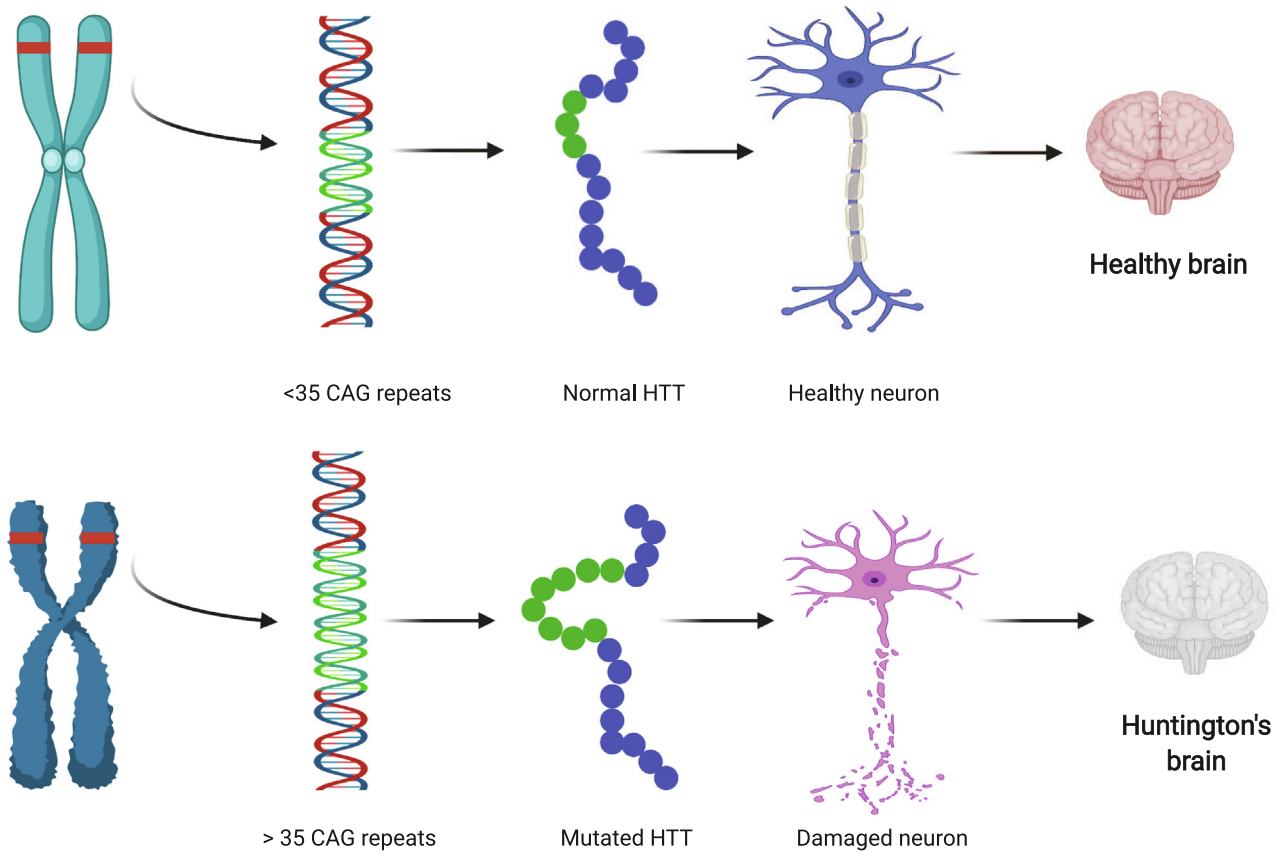

Fig. 5. Pathobiology of Huntington's diseases. The *HTT* gene contains a repeat of CAG codon coding for glutamine. If the repeat contains more than 35 repeats, it will lead to the development of Huntington's disease within a person's normal lifetime. This mutation results in the production of an altered protein leading to dysfunction and neuronal death in the brain's striatum region.

2006, 2006). Al-Dalahmah et al. (Diaz-Castro et al., 2019; Al-Dalahmah et al., 2020) used both RNA-Seq and snRNA-Seq to uncover HD-related expression profiles in the cingulate cortex area with a focus on astrocytes. The results showed the deregulation of genes involved in the immune system and neurotoxic, neuroprotective, and pan-reactive astrocytic genes in HD cases. However, snRNA-Seq showed reduced expression of Alzheimer's genes related to neurotransmitters functions in HD astrocytes, while upregulated genes were mostly enriched in metabolic pathways (Al-Dalahmah et al., 2020). Two HD astrocyte subclusters expressed high levels of metallothionein (*MT*) genes (markers for early reactive astrocyte clusters) and *GFAP*. The higher expression of *MT* genes in the astrocytic cluster Ast1 in the frontal cortex of human late-onset AD (Mathys et al., 2019) and the Brodmann area 4 of HD brain as well (Lin et al., 2016) was suggested to be a protective response to damage (van Lookeren Campagne et al., 1999; Al-Dalahmah et al., 2020). These studies demonstrated transcriptional changes of particular cell types such as reactive astrocytes in HD pathology (Al-Dalahmah et al., 2020).

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is the most common neuroinflammatory disease of young adults that attacks oligodendrocytes in the central nervous system (CNS) and causes demyelination and focal plaque formation (Fig. 6) (Dobson and Giovannoni, 2019). As plaques can form in different CNS areas in both white and gray matter, the MS clinical manifestation may be diverse (Compston and Coles, 2002; Klaver et al., 2013). The plaques are generally located around post-capillary venules and are characterized by the breakdown of the blood–brain barrier (BBB). Proinflammatory cytokines and chemokines produced by resident cells and endothelial cells dysregulate the BBB, resulting in migration of activated leukocytes into the CNS, oligodendrocyte loss, and demyelination (Filippi et al., 2018).

Although the molecular pathobiology of MS is still poorly understood, recent transcriptional studies have tried to shed the light on cell-type-specific mechanisms of MS lesions (Schirmer et al., 2019), understand the variability of MS symptoms (Jäkel et al., 2019), and characterize leukocytes in the cerebrospinal fluid of patients

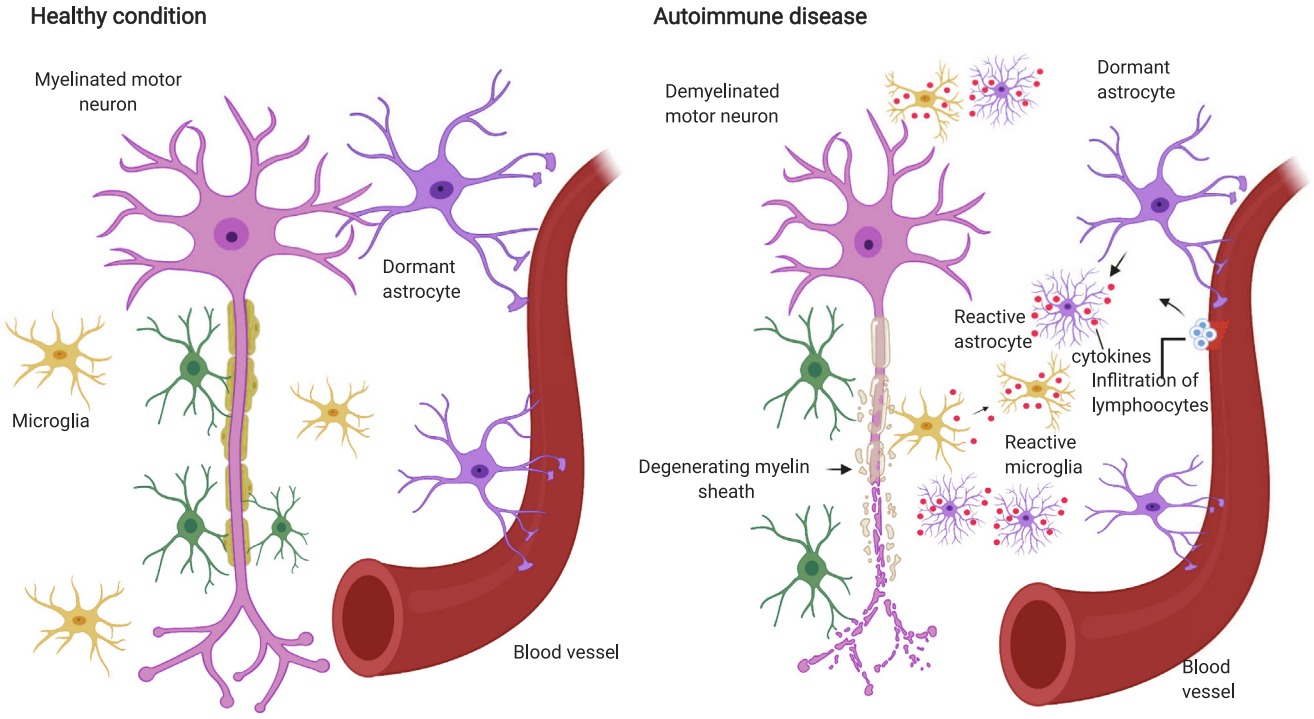

Fig. 6. Molecular mechanisms in the pathology of autoimmune diseases such as in MS and ALS. In the disease conditions: (1) Astrocytes are not able to support neuronal functions leading to neuronal excitotoxicity; (2) A damage to brain-blood-barrier leads to infiltration of lymphocytes and activation of inflammatory pathways; (3) The secretion of pro-inflammatory cytokines by activated microglia contributes to the development of an inflammatory milieu; (4) Dysfunctional microglia and astrocytes lead to degeneration of myelin sheath; and (5) synaptic failure, denervation and finally, muscle atrophy.

with MS (Schafflick et al., 2020). Transcriptional profiling of cortical gray matter (GM) and adjacent subcortical white matter (WM) lesion areas at different stages of inflammation and demyelination in MS patients (Schirmer et al., 2019) exhibited a selective decrease in numbers of CUX2-expressing excitatory neurons (ENs) of the upper layer (L2-L3) in MS patients with cortical demyelination, which was associated with the meningeal infiltration of plasma B cells expressing pro-inflammatory cytokines *IGHG1* and *MZB1*. In contrast, co-located inhibitory and other cortical EN subtypes were relatively preserved. Moreover, MS-associated genes showed the greatest differential expression in these cells, suggesting the cell-type vulnerability of L2–L3 ENs in MS lesion pathology (Schirmer et al., 2019). Oxidative stress, mitochondrial dysfunction, and cell death pathways were activated pathways in these cells. In contrast, mitochondrial energy consumption, glutamate signaling, and potassium or cation homeostasis were amongst downregulated gene ontology terms in L2–L3 ENs of patients with MS (Table S1) (Schirmer et al., 2019). The expression profiles of astrocytes were also affected by MS lesions; however, reactive astrocytes showed distinct expression patterns in cortical versus subcortical MS lesions. Oligodendrocytes and microglial cells were affected mainly at chronic active boundaries of subcortical MS lesions. For example, myelinating oligodendrocytes characterized by myelin gene expression and the transcription factor *ST18* showed molecular changes indicating cellular stress, degeneration, and iron overload (Schirmer et al., 2019). MS-microglia were enriched in transcripts encoding activation markers, complement factors, MHC class II-associated proteins, and lipid degradation proteins. These results show activation of immune system genes in particular cell types such as ENs and microglia in MS patients.

The study of different white matter areas of MS patients using snRNA-Seq (Jäkel et al., 2019) revealed transcriptional alterations in MS-oligodendrocytes including upregulation of several myelin protein genes. Similar upregulation of myelin genes was also observed in normal-appearing white matter (NAWM) of MS patients. An oligodendrocyte subcluster (Oligo1) was found depleted in MS, whereas the Oligo2, Oligo3, Oligo5, and immune oligodendroglia (ImOLG) clusters which were closely associated with microglia were enriched in MS. Also, fewer nuclei from OPCs were observed in all MS lesions and NAWM. Besides, oligodendrocyte subcluster 6 (Oligo6) was also highly reduced in MS patients both in lesions and in NAWM. These findings are in line with the concept that NAWM is indeed dysfunctional in MS (Pardini et al., 2017).

In contrast to brain tissue changes, single-cell maps of blood leukocytes (Schafflick et al., 2020) demonstrated no significant differences in blood cell composition in MS patients. However, MS altered the cell type composition of cerebrospinal fluid (CSF) such that all B lineage cell clusters, some natural killer cells (CD56dim NK1, CD56bri

NK2), CD8 naïve (CD8na) and regulatory T (Treg) cells, and a myeloid dendritic cell type (mDC1) were significantly increased. Disease-associated transcriptional changes were also evident in CSF cell clusters (Table S1). A greater proportion of genes was differentially expressed in blood than in CSF. This could be due to preferentially increased transcriptional and cell-type diversity in blood and CSF, respectively, suggesting compartment-specific disease mechanisms in MS (Schafflick et al., 2020). Focusing on CD4 T cells showed a reduction of a single memory-like cluster in MS patients' blood. Eventually, a CD4 subcluster of memory cells that expressed multiple genes associated with the cytotoxic function and pathogenicity, was more abundant in MS patients' CSF (Schafflick et al., 2020). These findings reflect the relationship between transcriptional changes in immune cells of CSF and brain tissues in MS.

AMYOTROPHIC LATERAL SCLEROSIS (ALS)

Amyotrophic lateral sclerosis (ALS) is recognized as a multisystem neurodegenerative disorder, with disease heterogeneity at the clinical, genetic, and neuropathological levels (Hardiman et al., 2017; Longo et al., 2017). The clinical characteristics of ALS usually include adult-onset focal muscle weakness and wasting, which tends to spread with disease progression. About 50% of patients suffer from extra-motor manifestations to some degree in addition to their motor problems (Phukan et al., 2007). About 15% of ALS cases represent an additional diagnosis of frontotemporal dementia, while nearly 40% of cases show mild behavioral and/or cognitive impairments (Neary et al., 1998; Phukan et al., 2007). Almost 20% of ALS cases have a familial background. The most frequent genetic factors in ALS include hexanucleotide expansions in chromosome 9 open reading frame 72 (C9orf72) and mutations in superoxide dismutase 1 (*SOD1*), TAR DNA-binding protein 43 (*TARDBP*), fused in sarcoma (*FUS*), and TANK-binding kinase 1 (*TBK1*) (Hardiman et al., 2017).

The most common neuropathological signature of ALS is the cytoplasmic aggregation of TDP-43 protein, encoded by the *TARDBP* gene, which is present in almost 95% of ALS cases (Neumann et al., 2006). TDP-43 is normally localized to the nucleus under basal conditions, but in ALS is mislocalized to the cytoplasm to form aggregates and become phosphorylated (Bertram and Tanzi, 2005; Chou et al., 2018). Other aggregating proteins, such as SOD1 and FUS, are found in patients bearing SOD1 and FUS mutations, respectively (Mueller et al., 2020). Patients with C9orf72 hexanucleotide repeat expansions have accumulations of dipeptide repeat proteins which are translated from the GGGGCC repeats, although this repeat is located in a non-coding region of the gene (Bertram and Tanzi, 2005).

Animal models and ALS patient-derived cell differentiation have been used to study the genetic components of ALS, particularly in motor neurons (Philips and Rothstein, 2015; Sances et al., 2016). Induced pluripotent stem cells (iPSC)-based disease modeling with a combination of genome engineering and RNA sequencing revealed activated *ERK* and *JNK* signaling as key players of neurodegeneration in SOD1-mutant motor neurons (MNs) (Chen et al., 2014). The AP1 complex member *JUN*, an *ERK/JNK* downstream target, was observed to be highly expressed in MNs compared with non-MNs, providing mechanistic insight into the specific degeneration of MNs (Chen et al., 2014). Liu et al (Liu et al., 2020) performed a scRNA-Seq analysis of samples obtained from the brainstem of wildtype and mutant SOD1 symptomatic mice, respectively. Cell types with more transcriptomic alterations in ALS mice were astrocytes > oligodendrocytes > Microglia > Ne urons2 > OPC > endothelial > Schwann Cells > Neurons1 > Ependymal. The most consistent DEG across cell types was *Sod1* (Liu et al., 2020), along with other genes (*e.g.*, *Malat1*, *mt-Cytb*, *mt-Rnr2*) that also showed significant differential expression across cell types. Several genes had a highly cell-type-specific differential expression (*e.g.*, *Tmem255a* in TG sensory cells and *Scn2a1* in MNs) indicating distinct changes in individual cell types (Liu et al., 2020). DEGs of individual cell populations revealed cell-type-specific alterations in numerous pathways, including previously known ALS pathways such as inflammation (in microglia), stress response (ependymal and an uncharacterized cell population), neurogenesis (astrocytes, oligodendrocytes, neurons), synapse organization and transmission (microglia, oligodendrocyte precursor cells, and neuronal subtypes), and mitochondrial function (uncharacterized cell populations). Other cell-type-specific processes altered in the SOD1-mutant brainstem include those from motor neurons (axon regeneration, voltage-gated sodium and potassium channels underlying excitability, potassium ion transport), trigeminal sensory neurons (detection of temperature stimulus involved in sensory perception), and cellular response to toxic substances (uncharacterized cell populations) (Liu et al., 2020). Whole transcriptome profiling of spinal cord ventral horns of postmortem ALS human donors using RNA-Seq showed 1160 deregulated genes, of which downregulated genes were neurons-related while upregulated genes were with glial origin involved in neuroinflammation (D'Erchia et al., 2017). Significant alterations of *SNAP25* and *STX1B* at both transcriptomic and proteomic levels were observed which leads to impaired synaptic function as a result of calcium elevation and glutamate excitotoxicity (D'Erchia et al., 2017).

In addition, molecular changes have been found in DAMs during neurodegenerative disease activation using the $SOD1^{G93A}$ mouse model of ALS (Chiu et al., 2013). Findings from this study displayed that $SOD1^{G93A}$ DAMs are not derived from infiltrating monocytes and that both potentially neuroprotective and toxic factors, including AD genes, are concurrently upregulated along with posttranscriptional regulation of microglia surface receptors and T cell-associated changes in the transcriptome.

Transcriptome profiling has also been utilized to investigate transcriptional changes at the single-cell resolution in ALS. Namboori et al. (2021) have also recently utilized scRNA-Seq of degenerating motor neurons derived from ALS patients to unravel key disturbed

pathways in ASL pathogenesis. Genes involved in synaptic structure, neuromuscular junction, neuronal cytoskeleton, and mitochondrial function showed significant downregulation in ALS-motor neurons (Namboori et al., 2021). However, interneurons did not show similar suppression of these homeostatic functions. Single-cell expression data also provided a context-specific transcriptional network relevant to ALS neurons. Master regulator analysis on this network identified core transcriptional factors driving the ALS disease signature. Specifically, suppression of *HOXA1* and *HOXA5* genes was correlated to synaptic dysfunction in ALS motor neurons (Namboori et al., 2021). This likely reflects that suppression of *HOX* genes may be a general phenomenon in SOD1 ALS (Ragagnin et al., 2019). Despite these important findings, more studies in the future are required to uncover the relatedness of transcriptional dysregulation in brain cell types and the mechanism of ALS pathogenesis.

To conclude, technologies such as scRNA-Seq and snRNA-Seq may be applied to achieve multiple goals in our understanding of brain health and disease. The data produced by these technologies could help re-evaluate hypotheses about differences between pre-defined sample groups at the single-cell level—regardless of their original classification. The application of these technologies for analysis of transcriptomic changes throughout disease stages in future human studies could provide detailed molecular vulnerability of specific cell types in the brain as an advantage over bulk RNA-Seq. Regarding this, scRNA-Seq databases will facilitate access to neurodegenerative-related findings (Jiang et al., 2020). Another goal could be providing a molecular atlas of the brain at the single-cell resolution which may enhance our knowledge of spatiotemporal structure and connectivity of cell types subpopulations in the brain. However, even though sc-RNA-Seq operates at the most basic level, mapping cell types and states at a specific level of resolution of interest may be challenging: Achieving the targeted level of resolution for the intended map of cells may require substantial methodological efforts.

Importantly, scRNA-Seq and snRNA-Seq technologies will facilitate the identification of sensitive diagnostic and prognostic biomarkers by investigating the discrepancies between patients and healthy individuals as well as using animal models. In this line, a great amount of research has been focused on understanding transcriptional changes in AD, and yet there is space for other neurodegenerative diseases to be more investigated. Eventually, the ultimate goal is to achieve efficient treatments by finding novel molecular targets in the CNS using scRNA-Seq and snRNA-Seq.

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COMPETING INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

All the authors made a significant contribution to the preparation of the manuscript. A.A. and I.S. researched the data and literature, contributed to writing and editing the manuscript. N.V-T., J.D.G., and A.N.C. contributed to reviewing and editing, and the discussion of the manuscript before submission.

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APPENDIX A. SUPPLEMENTARY DATA

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