When glia meet induced pluripotent stem cells (iPSCs)

Li Li¹, Yanhong Shi*¹

Division of Stem Cell Biology, Department of Developmental and Stem Cell Biology, Beckman Research Institute of City of Hope, Duarte, CA 91010, USA
Irell and Manella Graduate School of Biological Sciences, Beckman Research Institute of City of Hope, Duarte, CA 91010, USA

ARTICLE INFO

Keywords:
Glia
iPSC
Neurological diseases
Disease modeling
Astrocytes
Oligodendrocytes
Microglia

ABSTRACT

The importance of glial cells, mainly astrocytes, oligodendrocytes, and microglia, in the central nervous system (CNS) has been increasingly appreciated. Recent advances have demonstrated the diversity of glial cells and their contribution to human CNS development, normal CNS functions, and disease progression. The uniqueness of human glial cells is also supported by multiple lines of evidence. With the discovery of induced pluripotent stem cells (iPSCs) and the progress of generating glial cells from human iPSCs, there are numerous studies to model CNS diseases using human iPSC-derived glial cells. Here we summarize the basic characteristics of glial cells, with the focus on their classical functions, heterogeneity, and uniqueness in human species. We further review the findings from recent studies that use iPSC-derived glial cells for CNS disease modeling. We conclude with promises and future directions of using iPSC-derived glial cells for CNS disease modeling.

1. Introduction

Glia in general refers to non-neuronal cell types including astrocytes, oligodendrocytes, and microglia in the central nervous system (CNS). Other non-neuronal cell types also exist in the CNS, for example, ependymal cells that line up ventricles. Glial cells were traditionally viewed as cells that simply glue neurons and CNS tissues together. With increasing knowledge of their functions, each cell type is highly heterogeneous, exhibiting regionality and functional differences. It stands to reason that such abundant and highly specified cell populations play important roles in the physiology and pathology of the CNS. Indeed, the importance of glial cells to the CNS has been supported by numerous studies using animal models as well as human tissues (Sofroniew and Vinters, 2010; Bradl and Lassmann, 2010; Sominsky et al., 2018). Interestingly, it is shown that glial cells exhibit phenotypical and functional difference across species, and human glial cells possess unique features compared to other mammalian counterparts (Oberheim et al., 2006; Gosselin et al., 2017; Galatro et al., 2017). The divergence of human glial cells from other species may partially lead to the functional difference between the CNS of human and other species. This species difference poses challenges to study human CNS disorders using animal models.

Human induced pluripotent stem cells (iPSCs), which are generated by reprogramming of human somatic cells, provide a superior resource for researchers to derive cell types of interest and study diseases in a dish (Takahashi et al., 2007; Shi et al., 2017). The human iPSC platforms are particularly powerful to study neurological diseases due to the uniqueness of the human CNS system and the challenges to access and maintain human CNS tissues and cells in vitro. Because glial cells play an important part in the pathogenesis of neurological diseases, here we review the key properties of glial cells and the contribution of iPSC-derived glial cells to our understanding of CNS diseases (Fig. 1). We will also discuss the limitations to overcome when using iPSC-derived glial cells to study CNS diseases based on current knowledge of glial biology.

2. Glial cell function, heterogeneity, and their uniqueness in human

Glia in the human CNS maintain tissue homeostasis, form myelin sheaths, and respond to injuries and pathogens. The dysfunction of glial cells due to genetic mutations, aging or exogenous insults contributes to various CNS diseases. In this section, we will provide an up-to-date overview of the functions of the three major glial cell types (astrocytes, oligodendrocytes and microglia) under physiological conditions, their heterogeneity and species-specific features.

2.1. Astrocytes

Astrocytes are the most abundant cell type and involved in various
functions in the CNS. They are highly heterogeneous in terms of morphology, molecular profiles, and functions depending on their location as well as reactive states. Grey matter astrocytes, also named protoplasmic astrocytes, exhibit highly branched morphology and are in close contact with neurons and blood vessels; white matter astrocytes, also named fibrous astrocytes, have less branches and simpler processes and are in contact with oligodendrocytes and axons in the nodes of Ranvier (Sofroniew and Vinters, 2010). Astrocytes switch from a non-reactive state to a reactive state in response to stimuli. Reactive astrocytes respond differentially to different insults and can be divided into neurotoxic A1 and neuroprotective A2 subtypes (Liddelow et al., 2017; Clarke et al., 2018). Astrocyte reactivity is accompanied by a dynamic yet reversible change in gene signature, morphology, and functions (Anderson et al., 2014).

Astrocytes play important roles in synapse formation, clearance and function (Allen and Eroglu, 2017; Pfeifer and Barres, 1997). The support of synapse formation by astrocytes has been observed across different species (Colon-Ramos et al., 2007; Muthukumar et al., 2014; Ullian et al., 2001), in different subtypes of neurons (Elmariah et al., 2005; Cuevas et al., 2005; Cao and Ko, 2007), and through different mediators, for example, cholesterol (Goritz et al., 2005; Mauch et al., 2001), thrombospondins (Christopherson et al., 2005; Kucukdereli et al., 2011), and Chrdl1 (Blanco-Suarez et al., 2018). Astrocytes can phagocytose synapses directly through the phagocytic receptors Merk and Megf10 to avoid excessive synapse accumulation (Chung et al., 2013) or signal microglia to clear synapses through molecules such as TGFβ and IL-33 (Vainchtein et al., 2018). Astrocytes form tripartite synapses as an addition to pre- and post-synaptic neuronal communications to allow bidirectional information flow between astrocytes and synapses (Arnaque et al., 1999; Perea et al., 2009). Astrocytes generate intracellular Ca2+ oscillations in response to synaptic neurotransmitters and release neuroactive molecules, such as glutamate, acetylcholine, adenosine, ATP, and γ-aminobutyric acid (GABA), through vesicle and lysosomal exocytosis (Perea et al., 2009). These molecules in turn influence neuronal and synaptic activity.

Astrocytes act as sensors for various metabolic signals, such as glutamate, O2, CO2, glucose and endocrine hormones (Angelova et al., 2015; Garcia-Caceres et al., 2016; Marina et al., 2018). Glutamate is one major excitatory neurotransmitter released from neuronal presynaptic terminals (Rose et al., 2017). The accumulation of glutamate at the synapse space leads to neuronal injury through Ca2+ influx and subsequent nitric oxide synthesis, the generation of free radicals, the activation of autophagy/lysosomal pathways, and programmed cell death (Dong et al., 2009). Astrocytes express EAATs (excitatory amino acid transporters), which allows glutamate uptake from the extracellular space (Rose et al., 2017), protecting the brain from glutamate-induced excitotoxicity.

The endfeet of astrocytes attach to the microvessel wall in the brain, named the blood-brain barrier (BBB), which selectively permits or excludes molecules in the blood stream to enter the brain. Specialized features in astrocyte endfeet, such as high density of the water channel aquaporin 4 (AQP4) and the Kir4.1 K+ channel, are involved in ion and fluid regulation of the brain (Daneman and Prat, 2015; Verkman, 2002).

![Fig. 1. When glial cells meet iPSCs. Glial cells mainly include three cell types, astrocytes, oligodendrocytes and microglia. Human glial cells play critical roles in brain functions, are highly heterogeneous, and possess species-specific features. Human iPSCs generated from somatic cell reprogramming can be differentiated into glial cells for disease modeling.](image-url)
Although mouse models contribute tremendously to our understanding of astrocytes, mouse astrocytes differ from human astrocytes in various ways. Astrocytes in human brains exhibit more complex morphology (Oberheim et al., 2006) and possess a set of genes uniquely expressed compared to murine astrocytes (Zhang et al., 2016). Functionally, human astrocytes can generate calcium waves in response to glutamate, whereas mouse astrocytes cannot (Zhang et al., 2016), which may contribute to behavioral differences between species (Windrem et al., 2017; Chen et al., 2015). Moreover, human astrocytes can grow better than mouse astrocytes after being transplanted into mouse brains. Finally, they gradually replace endogenous astrocytes in mouse brains and improve cognitive functions of the recipient mice (Windrem et al., 2014).

### 2.2. Oligodendrocytes

Oligodendrocytes are the cell type that perform myelination in the CNS. Oligodendrocytes are developed from PDGFβRα⁺ and NG2⁺ oligodendrocyte progenitor cells (OPCs) (Rivers et al., 2008). OPCs can differentiate into O4⁺ late OPCs (late OPCs) or preoligodendrocytes (Bansal et al., 1989; Sommer and Schachner, 1981), followed by O1⁺ premyelinating OLs or immature oligodendrocytes (Sommer and Schachner, 1981). Immature oligodendrocytes can terminally differentiate into mature myelinating oligodendrocytes, which are marked by the expression of myelin proteins, such as MBP and PLP1 (Emery et al., 2009; Goldman and Kuypers, 2015). The process of myelin wrapping around axons is called myelination. At least four important steps occur during the process of myelination: 1) selection of axons by oligodendrocytes for wrapping and initial oligodendrocyte-axon contact; 2) stabilization of oligodendrocyte-axon contact and formation of nodes of Ranvier; 3) myelin wrapping of axons and compaction; and 4) longitudinal extension of myelin segments in response to postnatal axon extension (Nave and Werner, 2014).

The impacts of myelination on CNS functions are beyond a passive insulator. The speed of signal conduction along axons is tuned by subtle structural changes of myelin, for example, myelin sheath thickness and the length of myelin segments. This fine adjustment of myelin parameters is crucial for motor skill establishment and cognitive function (Liu et al., 2012; Makinodan et al., 2012). In addition, oligodendrocytes provide metabolic products, such as lactate and pyruvate, to support mitochondrial functions and neurotrophic factors to maintain the integrity of axons (Philips and Rothstein, 2017; Lee et al., 2012; Funschilling et al., 2012).

Emerging evidence supports the heterogeneity of OPCs and oligodendrocytes. At the OPC stage, they differ in terms of origin (dorsal versus ventral), rate of self-renewal (white-matter OPCs proliferate faster than grey-matter OPCs), differentiation potential (white-matter OPCs differentiate more efficiently than grey-matter OPCs), and regenerative capacity (dorsal OPCs make more contribution to remyelination than ventral OPCs, however, under aging, their regeneration properties are reversed) (Zhang et al., 2016). In addition, Marques et al. analyzed mouse oligodendrocytes from different brain regions using single-cell RNA-sequencing (scRNA-seq) and identified six subgroups of oligodendrocyte subpopulations (Marques et al., 2016). Single-cell electrophysiological recordings also demonstrated ion channel changes of OPCs between different brain regions and ages (Spitzer et al., 2019).

Studies of human oligodendrocytes and OPCs are relatively rare due to technical challenges of obtaining and processing human postmortem brain tissues. Nevertheless, Jäkel et al. recently performed single-nucleus RNA sequencing (snRNA-seq) of white matter brain tissues from multiple sclerosis patients and unaffected controls (Jäkel et al., 2019) and identified 7 subclusters of oligodendrocytes with different transcriptome signatures, supporting the heterogeneity of human oligodendrocytes. The comparison between multiple sclerosis and control snRNA-seq data indicates mature oligodendrocytes also contribute to remyelination in humans, which is different from rodents, in which the recruitment of OPCs followed by the induction into oligodendrocytes is the only driver for remyelination. Retrospective carbon-14 birth-dating analysis of oligodendrocytes in human brains also suggests that mature oligodendrocytes contribute to myelin remodeling rather than newly generated oligodendrocytes (Yeung et al., 2014).

### 2.3. Microglia

Microglia are the innate immune cells of the CNS. Microglia originate from a subset of primitive myeloid progenitors traveling to neural tube at the yolk sac stage during primitive hematopoiesis from approximately embryonic day 8 (EB) in mouse (Ginhoux et al., 2010) and gestation week 4.5 in human (Monier et al., 2007; Monier et al., 2006; Verney et al., 2010). Microglia progenitors proliferate and mature within brain tissues (Erny et al., 2015), a process tightly regulated by neural cues in the CNS (Bennett et al., 2018) and brain-gut interactions (Erny et al., 2015; Matcovitch-Natan et al., 2016).

As innate immune cells, microglia express pattern recognition receptors, such as the Toll-like receptor 4 (TLR4) (Colonna and Butovsky, 2017), the activation of which triggers downstream signaling pathways, e.g. the NF-κB pathway, and the secretion of pro-inflammation cytokines and complement factors. These secreted molecules recruit other immune cells, such as T cells and macrophages, from the blood stream into the CNS and continue immune reactions under pathological conditions (Colonna and Butovsky, 2017).

Microglia are regulators for brain functions at both embryonic and postnatal stages. During development, microglia phagocytose redundant cells to ensure the appropriate quantity of cell types at the right locations (Cunningham et al., 2013; Wakselman et al., 2008; Hoshiko et al., 2012). They can precisely target and phagocytose unwanted synapses tagged by the complement factor C1q and C3 (Bialas and Stevens, 2013). Refinement of synapses by microglia persists throughout the lifetime, which is important for neuronal plasticity (Stevens et al., 2007). Microglia also engulf myelin debris after brain injury and promote remyelination (Lampron et al., 2015).

Depending on the immune reaction status, microglia are categorized using the paradigm of macrophages, M1 as neurotoxic and M2 as neuroprotective (Cherry et al., 2014). However, with increasing knowledge about the response of microglia and macrophage to immune stimuli, the status of microglia is more complicated beyond the scope of the M1/M2 nomenclature (Michell-Robinson et al., 2015). They may respond differently to different molecular constituents, such as LPS, IFNγ, and interleukin cytokines (Mantovani et al., 2004; Edwards et al., 2006). With the progress of single cell technology, such as scRNA-seq, microglia have been shown to be diverse in terms of their developmental timing, turnover rate, response to insults, and regional differences. Li et al. performed deep scRNA-seq of microglia isolated from different regions of mouse brains and observed a higher level of heterogeneity at the early postnatal stage than adulthood (Li et al., 2019). Very recently, Masuda et al. comprehensively characterized the subtypes of microglia at different regions in the CNS during development and diseases (Masuda et al., 2019) and found both spatial and temporal heterogeneity exist in microglia populations, with 10 clusters showing distinct transcriptional profiles, some of which mainly consisted of the embryonic stage and others of the postnatal stage. The authors also compared the human microglia data with the mouse microglia data, finding overall similarities between human and mouse microglia, but with non-negligible species differences.

### 3. Contribution of iPSC-derived glial cells to disease modeling

The human CNS diverges from the CNS of the other species in various ways, but current findings of developmental biology and disease pathology have mainly relied on animal models. To overcome species differences, human brain tissues (mainly postmortem tissues)
have been used as a supplement to confirm findings from animal models. Most human brain tissues represent end-stage diseases and undergo extensive processing before analysis, which prevents us from understanding the initiation and progression of disease conditions that are critical for disease prevention and intervention at the early stage. The emergence of the iPSC technology has added to the toolbox for disease study due to the unique advantages of iPSCs. iPSCs maintain genetic features of the parental somatic cells and mimic embryonic stem cells (ESCs) in many aspects, such as potent proliferation and relatively easy manipulation of the genome. These advantages make iPSCs a powerful tool for human disease modeling, especially neurological disease modeling, for which human tissues are not easily accessible.

With the advances of differentiation protocols, iPSC-derived astrocytes, oligodendrocytes, and microglia have been applied to study various CNS diseases and answered questions not possible using animal models. Below, we discuss cellular models using human iPSC-derived glial cells and their contribution to our understanding of CNS diseases. However, iPSC-based modeling systems are not perfect, and like any other models, caution is needed when interpreting the results. We will thus discuss how to overcome these obstacles as well.

### 3.1. Astrocytes

Astrocyte dysfunction plays a role in a wide range of CNS diseases, not only neurodegeneration, but also neurodevelopmental and neuropsychiatric diseases. For example, astrocyte abnormalities, especially reactive gliosis, have been identified in postmortem patient brain tissues and animal models of Alzheimer’s disease (AD), Parkinson’s disease (PD) and Huntington’s disease (Phatnani and Maniatis, 2017). Reactive astrocytes could also lead to gliosis formation after spinal cord injury or brain trauma (Burd et al., 2014; Sofroniew, 2014). In addition, mutations of GFAP, a gene predominantly expressed in astrocytes, are sufficient to cause the fatal disorder Alexander disease (AxD) (Messing et al., 2012; Olabarria and Goldman, 2017). Because astrocytes interact with all the major cell types within the CNS, their abnormalities could affect various functions of other cell types and contribute to diseases. Importantly, iPSC-derived astrocytes allow researchers to uncover the roles of astrocytes in the pathogenesis of diseases that remain challenging to understand due to the lack of reliable animal models or the absence of disease-relevant phenotypes in animal models. Neurodegenerative diseases, such as AD and PD, are of particular interest, because it has been challenging to dissect the complications of these diseases and develop effective treatments using traditional models. Rare neurodevelopmental diseases are also being more studied using human iPSC models because of the easy access of cellular resources derived from iPSCs and the flexibility of genome editing of iPSCs (summarized in Table 1 by disease type).

#### 3.1.1. Physiological models

Progress has been made in studying the role of astrocytes in disease pathogenesis due to the development of astrocyte differentiation protocols from iPSCs (Li et al., 2018a; de Majo et al., 2020). Different cellular models including human iPSC-derived astrocytes have been established to meet different needs, including astrocyte-only cultures (Tyzack et al., 2017; Perriot et al., 2018; Jones et al., 2017; McGivern et al., 2013), astrocytes co-cultured with other neural cell types (Du et al., 2018; di Domenico et al., 2019; Thomas et al., 2017; Oksanen et al., 2017; Serio et al., 2013; Russo et al., 2018; Barbar et al., 2020), astrocyte-containing 3D organoids (Sloan et al., 2017), and mouse models in which human astrocytes are transplanted (Windrem et al., 2014).

These models have revealed novel links between disease-causing gene mutations and astrocyte functions, such as inflammatory cytokine secretion (e.g. IL1-b, IL6, TNF-a) (Perriot et al., 2018; Jones et al., 2017), decreased neurotrophic factor secretion (e.g. GDNF) (McGivern et al., 2013), and neuroprotective signaling pathways (e.g. STAT3) (Tyzack et al., 2017). In addition, astrocyte-neuron co-culture models have revealed a broad range of astrocytic effects on neuronal properties, for example, neurogenesis, neuronal survival, calcium signals and mitochondrial function in neurons (Du et al., 2018; di Domenico et al., 2019; Thomas et al., 2017; Oksanen et al., 2017; Serio et al., 2013; Russo et al., 2018; Barbar et al., 2020). 3D brain organoids are comprehensive models that have enriched the toolbox for studying neurological diseases. Astrocytes are an essential component in brain organoids and have been shown to mimic fetal astrocyte features at the molecular and functional levels (Sloan et al., 2017).

Interactions between astrocytes and other glial cells are relatively less studied in animal- and iPSC-based models. Modeling AxD is a good demonstration of the necessity of using iPSCs to improve our understanding on astrocyte interactions with other glial cells. AxD is a type of leukodystrophy disease caused by GFAP mutations. It is defined as a primary astrocyte disease based on its pathological hallmark of AxD, the Rosenthal fiber, which is only identified in astrocytes from patient brain tissues. Our group has recently established a human iPSC-based modeling system consisting of human iPSC-derived astrocytes and OPCs as well as oligodendrocytes to study astrocyte-OPC and astrocyte-oligodendrocyte interactions in the context of AxD (Li et al., 2018b; Sofroniew, 2018), which demonstrated a novel regulatory role of astrocytes in regulating OPC proliferation and oligodendrocyte myelination.

Additionally, emerging evidence suggests astrocytes alone could have important functions in memory formation through calcium signaling (Adamsky et al., 2018). Overall, it would be interesting to study their roles in human species and disease context; however, it is challenging to confirm the functional output without an in vivo environment. To resolve this issue, studies by Windrem et al. and Qian et al. combined the advantages of iPSCs and mouse models (Windrem et al., 2017; Windrem et al., 2014; Qian et al., 2017) to establish glial chimeras by transplanting human iPSC-derived glial progenitor cells into mouse brains, demonstrating the effects of human astrocytes on animal behaviors, such as memory and sleep.

#### 3.1.2. Neurodevelopmental and neuropsychiatric disorders

Using iPSC-derived astrocyte models, researchers have looked into the roles of astrocytes in neurodevelopmental and neuropsychiatric diseases. For example, Thomas et al. studied Aicardi-Goutières syndrome (AGS), an autoimmune disease that affects neurodevelopment, using iPSC-derived neural cells (Thomas et al., 2017). Dysfunction of three-prime repair exonuclease 1 (TREX1) in AGS causes an accumulation of extrachromosomal DNA with unknown mechanisms and impairs the intellectual and physical functions of the patients (Stetson et al., 2008). Using iPSC-derived neurons, astrocytes and 3D brain organoids, Thomas et al. identified endogenous Long Interspersed Element-1 retrotransposons as a major source of extranuclear DNA accumulation in neurons, astrocytes and neural progenitor cells (NPCs). They also found that TREX1-deficient human astrocytes could secrete neurotoxic type I interferons, which further exacerbate the neurotoxicity. Barbar et al. identified CD49f as a novel marker for functional astrocytes derived from iPSCs (Barbar et al., 2020). The same study showed that CD49f* astrocytes could exhibit an A1 reactive state in response to inflammatory cytokine stimulation and cause neurotoxicity.

We have used a co-culture system consisting of human iPSC-derived astrocytes and OPCs as well as oligodendrocytes in combination with postmortem patient brain tissues to study AxD. We demonstrated that the AxD iPSC-derived astrocytes could inhibit OPC proliferation by secreting more CHI3L1 (Chitinase-3-like protein 1; also known as YKL-40), a neuroinflammatory molecule, which in turn contributes to decreased myelination. Interestingly, the expression patterns of CHI3L1 differ between mouse and human according to RNA-seq analysis of different brain cell types in these two species. In human, CHI3L1 is predominantly expressed in mature astrocytes. However, it is detected...
| Disease | Disease features of interest | Disease iPSC characteristics | Cellular model | Major findings |
|---------|-----------------------------|-----------------------------|---------------|---------------|
| Aicardi-Goutières syndrome (AGS) | Long Interspersed Element-1 retrotransposons is a major source of extranuclear DNA accumulation; TREX1-deficient astrocytes | TREX1-mutant human ESCs generated by CRISPR and TH1X-mutant iPSCs from AGS patients | Astrocytes, neurons and brain organoids | Increased extranuclear DNA accumulation and increased neurotoxicity in AGS astrocytes compared to TREX1-deficient astrocytes |
| Alzheimer's disease (AD) | Astroglial contribution to AD (Jones et al., 2017) | iPSCs from patients with FAD carrying M146L mutation in PSEN1 and ApoE4 | Astrocyte-neuron co-cultures | Altered mitochondrial metabolism followed by an increase in extracellular ATP release leading to attenuated calcium wave propagation |
| | | | | | |
| | | | | |
| Amyotrophic lateral sclerosis (ALS) | Effects of GFAP mutation on astrocytes (Kondo et al., 2016) | SOD1 D90A mutant iPSCs from ALS patients and isogenic SOD1 wild type control iPSCs | Astrocyte-OPC or astrocyte-oligodendrocyte co-cultures | Altered cholesterol metabolism and impaired Aβ clearance in APOE4 iPSC-derived astrocytes |
| | | | | | |
| | | | | |
| | | | | |
| | | | | |
| Schizophrenia (SCZ) | Effect of astrocyte from sALS patients on motor neurons (Qian et al., 2017) | iPSCs from LRRK2 G2019S mutant fPD patients | Astrocyte-neuron co-cultures | Impaired extracellular ATP release leading to attenuated calcium wave propagation |
| | | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
Table 2

Selected studies investigating neurological diseases using iPSC-derived oligodendrocytes.

| Disease | Cellular model | Major findings |
|---------|----------------|----------------|
| Amyotrophic lateral sclerosis (ALS) | Oligodendrocytes | Mutant superoxide dismutase 1 (SOD1) oligodendrocytes induce wild type motor neuron death. |
| Pelizaeus–Merzbacher disease (PMD) | Oligodendrocytes | Decreased secretion of CHI3L1 from AxD-iPSC-derived astrocytes inhibits OPC proliferation. |
| Alexander disease (AxD) | Oligodendrocytes | Variable oligodendrocyte phenotypes across PLP1 point mutation, duplication or deletion. |
| Schizophrenia (SCZ) | Glial progenitor cells | Fewer oligodendrocytes in SCZ brains concordant with abnormalities seen by in vivo brain MRI studies. |
| Neurodegenerative disorders | Oligodendrocytes | Mutant OLs with abnormal morphology, impaired viability, and defective maturation to OLs. |

3.1.3. Neurodegenerative disorders

Neurodegenerative diseases, such as AD and PD, are under the spotlight of iPSC modeling because of the increasing recognition of human and rodent model differences. One PD model included both iPSC-derived astrocytes and dopaminergic neurons with or without the G2019S mutation in LRRK2 (leucine-rich repeat kinase 2), one of the most common disease-causing mutations for familial PD (d’Domenico et al., 2017). Finally, the human-mouse glial chimera model mentioned in Section 3.1.1 was applied to study the contribution of astrocytes to the pathogenesis of schizophrenia (SCZ). The chimera mice exhibited delayed astrocyte maturation and abnormal behaviors, including excessive anxiety, antisocial traits and disturbed sleep (Windrem et al., 2017).

3.2. Oligodendrocytes

As the only cell type that performs the function of myelination, oligodendrocyte dysfunction is associated with a variety of CNS diseases. Oligodendrocyte dysfunction could be due to gene mutations interrupting oligodendrocyte lineage differentiation, proliferation or myelination and is mainly seen in leukodystrophy diseases such as Canavan disease (CD) caused by ASPA gene mutations (Matalon et al., 1988; Surendran et al., 2003), Krabbe Disease (KD) caused by GLC mutations (Wenger et al., 2000), Pelizaeus-Merzbacher disease (PMD) caused by PLP1 gene mutations (Inoue, 2005; Hudson et al., 1989), and Vanishing White Matter disease (VWM) caused by EFI2B gene at similar levels in both astrocytes and OPCs in mouse (Zhang et al., 2016). This may indicate different roles of CHI3L1 in human and mouse brains that need to be considered when studying its function using mouse models. AxD has also been studied using iPSC-derived astrocytes alone by other groups. AxD-iPSC-derived astrocytes exhibit an increased secretion of inflammatory factors and impaired extracellular ATP release (Jones et al., 2018; Kondo et al., 2016). Astrocytes derived from neuropsychiatric patient iPSCs, such as Autism spectrum disorder (ASD) and Rett Syndrome (RTT), showed adverse effects on neuronal functions, such as neuronal development and survival, neurite length, and synapse formation (Krasovska and Doering, 2018; Williams et al., 2014). Finally, the human-mouse glial chimera model mentioned in Section 3.1.1 was applied to study the contribution of astrocytes to the pathogenesis of schizophrenia (SCZ). The chimera mice exhibited delayed astrocyte maturation and abnormal behaviors, including excessive anxiety, antisocial traits and disturbed sleep (Windrem et al., 2017).
| Disease | Disease features of interest | Disease iPSC characteristics | Cellular model | Major findings |
|---------|----------------------------|----------------------------|----------------|----------------|
| AD | Morphological changes of APOE4 microglia with reduced Aβ phagocytosis in vitro | PSEN1ΔE9, and APPswe iPSCs; impaired phagocytosis, migration and metabolic activity in vitro | Microglia in vitro | Increased synapse elimination by SCZ microglia |
| AD | Effects of AD risk factor APOE4 on multiple-neural cell types, Aβ plaques, and microglia | Isogenic APOE3 and APOE4 iPSCs generated by CRISPR | Microglia in vitro | Microglia acted as the reservoir for ZIKV that invaded the neural tissue and initiated the infection |
| AD | Effects of AD risk factor APOE4 on multiple-neural cell types, Aβ plaques, and microglia | Isogenic TREM2 R47H mutant generated by CRISPR | Microglia in vitro | Microglia infected with MR766 strain of ZIKV and integrated into brain organoids |
| NHD | Role of TREM2 mutation in NHD (Garcia-Reitboeck et al., 2018) | iPSCs from patients with TREM2 mutation (NHD) | Microglia in vitro | Microglia infected with MFT766 strain of ZIKV and integrated into brain organoids |
| SCZ | Synapse density reduction in SCZ brains (Sellgren et al., 2019) | iPSCs from healthy individuals | Microglia in vitro | MicrogliainfectedbyZIKVtransmittedthevirustoNPCsand increased NPC apoptosis |
| ALS | Oxidative stress due to a high metabolic rate, high iron level and low antioxidant glutathione level during maturation and myelination (Giacci and Fitzgerald, 2018; Butts et al., 2008) | ES-derived OPCs and the competitive advantage of dorsal OPCs over ventral OPCs (Kim et al., 2019). Besides using the organoid approach to study oligodendrocyte lineage and model diseases, Windrem et al. combined the advantages of human iPSC-based disease models and animal models in their study of SCZ (Windrem et al., 2017). They transplanted human iPSC-derived glial progenitors into Shiverer mice that lack endogenous myelin and generated glia chimeras of human and mouse. When comparing chimeras that had SCZ or control iPSC-derived glia progenitors transplanted into Shiverer mice, the authors observed cell differentiation defects, hypomyelination and behavioral abnormalities in the SCZ chimeras, indicating the contribution of glial cells to the SCZ pathogenesis (Windrem et al., 2017). |
| MS | The role of microglia in CNS diseases has been increasingly appreciated. As the resident immune cells in the brain, they are the mediators of inflammatory responses in the brain to various insults. Inflammation...
is almost a ubiquitous phenotype observed in neurodegenerative diseases, such as AD and PD, as well as CNS injuries, for example, cerebral ischemia and traumatic brain injury (Skaper et al., 2018). Over-activation of microglia has been observed in these diseases. Therefore, it is critical to study how human microglia respond to environmental changes and contribute to disease initiation and progression. Besides immune functions, microglia also sculpt neural circuits by engulfing synaptosomes and cleaning protein debris through phagocytosis. Because over-excitation is a key phenotype of many psychiatric diseases (Mondelli et al., 2017), such as SCZ and ASD, the dysfunction of microglia phagocytosis could be important for the pathogenesis of psychiatric disorders. We will review the application of iPSC-derived microglia for disease modeling below (summarized in Table 3).

3.3.1. Physiological models
To study human microglial functions and their roles in diseases in vitro, multiple protocols have been developed to derive microglia-like cells (iMGs) from human ESCs and iPSCs (Abud et al., 2017; Douvaras et al., 2017; Muffat et al., 2016; Pandya et al., 2017). iMG-based models have been applied to study the phagocytic capability of the cells for synapses in vitro (Lin et al., 2018; Konttinen et al., 2019b). There are also studies focusing on the roles of microglia in early neural development by co-culturing iMGs with NPCs or integrating iMGs into 3D brain organoids (Mesci et al., 2018; Muffat et al., 2018). It is noteworthy that iMGs still differ from primary microglia in certain aspects. Microglia as an immune cell type are sensitive to in vitro culture conditions, therefore, microglia (both iMGs and primary microglia isolated from tissues) are prone to act in the active state rather than the resting state. Transcriptomic analysis of microglia cultured in vitro demonstrated that the cells exhibit significant changes of gene expression levels when cultured in vitro for 6 h, including the down-regulation of microglia cell-type-specific genes, such as TNEM119 and P2RY12, and up-regulation of microglia activation genes (Gosselin et al., 2017; Bohlen et al., 2017). These limitations have motivated studies aimed at maintaining ground-state microglia by transplanting iPSC-derived intermediate progenitors into mouse brains. These iMG progenitors were transplanted into NOD-scid gamma (NSG) mice expressing human cytokines IL3, SCF, GM-CSF and CSF1 (Svoboda et al., 2019). Under the in vivo environment, the microglia precursors not only matured into microglia that possess a similar morphology and gene expression signatures as primary microglia, but they also showed cellular heterogeneity, as evidenced by scRNA-seq analysis.

3.3.2. Neurodegenerative and neuropsychiatric disorders
Microglia have been shown to play significant roles in neurodegenerative and neuropsychiatric diseases (Colonna and Butovsky, 2017; Mondelli et al., 2017). A study by Lin et al. examined multiple cell types derived from AD patient iPSCs carrying the APOE4 allele including iMGs and observed morphological changes of the ApoE4 microglia that is associated with Aβ phagocytosis (Lin et al., 2018). Konttinen et al. observed additional functional changes of ApoE4 iMGs, including impaired phagocytosis, migration and metabolic activity and increased cytokine secretion (Konttinen et al., 2019b). Hasselmann et al. transplanted iPSC-derived hematopoietic progenitors into mouse brains and observed microglia generation in vivo, which exhibited different gene signatures compared to mouse microglia in response to Aβ plaques (Hasselmann et al., 2019). Garcia-Reiboèbe et al. modeled an early-onset dementia, named Nasu-Hakola disease (NHD), caused by homozygous TREM2 mutation using iMGs (Garcia-Reiboèbe et al., 2018). They found that the TREM2 mutation could reduce iMG survival and phagocytosis of apoptotic bodies. Sellgren et al. investigated the role of microglia in SCZ using iMGs and found an increased elimination of synapses in patient iMGs that could be reduced using the antibiotic minocycline (Sellgren et al., 2019).

3.3.3. Environmental factors
iMGs have also been used to investigate the effects of environmental factors, such as Zika virus (ZIKV), on neural function and development. Muffat et al. investigated the infection of ZIKV on different neural cell types using iPSC-derived cells, including NPCs, astrocytes, and microglia (Muffat et al., 2018). They identified microglia as the reservoir for ZIKV that invaded the neural tissues and initiated the infection, providing a mechanism for how ZIKV occurs at the early stage of pregnancy. Mesci et al. also investigated how the antiviral immune response of microglia could affect NPCs using a novel microglia-NPC co-culture system. They found microglia infected by ZIKV could transmit the virus to NPCs and increase NPC apoptosis. Importantly, they also demonstrated that Sofosbuvir (SOF), an FDA-approved drug against Hepatitis C infection, was able to reduce ZIKV-induced NPC death (Mesci et al., 2018).

4. Future directions
Glial cells derived from human iPSCs have expanded our knowledge about their importance in physiology and contributions to CNS diseases, especially in diseases that are challenging to study using animal models. Continuous efforts have been made to optimize in vitro culture conditions in order to better mimic the in vivo environment. An emerging new tool to achieve this goal is brain organoids, which are pluripotent stem cell-derived self-organized architectures composed of progenitors, neurons, and glial cells (Lancaster et al., 2013; Pasca et al., 2015; Qian et al., 2016; Quadrato et al., 2015; Camp et al., 2015; Cugola et al., 2016). Brain organoids mimic many aspects of brain development, such as the neuroepithelial structure and molecular and cellular developmental trajectories (Trujillo et al., 2019), therefore, they are a promising tool for researchers to study multiple neural cell type interactions under physiological and disease conditions. A number of studies have described protocols to generate astrocytes, oligodendrocytes and microglia in brain organoids and studied their features during development or under disease conditions (Sloan et al., 2017; Martin et al., 2019; Madhavan et al., 2018). Sloan et al. took advantage of the 3D organoid structure to study the transcriptional and functional maturation of human astrocytes, which has been challenging due to the difficulty of obtaining human brain tissues at early and late gestation stages (Sloan et al., 2017). They observed transcriptional maturation from fetal to mature astrocytes over time, accompanied with functional alterations such as a decrease in the capacity to phagocytose synapses. These findings will facilitate the application of iPSC-derived astrocytes to development and disease research. Because microglia originate from the hematopoietic system, which is from the mesodermal layer of the embryo, whereas other CNS neural cell types originate from the neuroectoderm layer, the majority of brain organoid protocols do not generate microglia. However, the flexibility of in vitro cultures allows integrating microglia into brain organoids (Lin et al., 2018; Abud et al., 2017) to study neuro-immune interactions. A recent study has described a protocol that generates cells with typical microglial molecular phenotype, morphology, and function in brain organoids (Ormel et al., 2018). As another important component of the brain, the vasculature can be integrated into brain organoids in vivo (Mansour et al., 2018) and in vitro (Pham et al., 2018), which improves the nutrient supply and growth potential of brain organoids and enables modeling interactions between the neural and blood systems.

Glial cells are highly heterogenous. One of the biggest challenges of using iPSC-derived glial cells to study CNS diseases is to accurately generate spatially and temporally defined glial subtypes. It is widely acknowledged that specific neuronal subtypes are needed to study diseases that affect corresponding brain regions. Accordingly, glial subtypes are also critical to accurately study the role of the glial type in disease. Due to the heterogeneity, different subtypes of glial cells possess different transcript profiles and perform distinct functions in their
territories at specific developmental timing. White-matter disease models need white-matter glial cells to study glial cell interactions with each other and neurons. Similar to the establishment of cell-type differentiation protocols, which benefit from developmental biology, the progress of generating subtypes of glial cells from iPSCs will also greatly rely on the progress of glial biology through in vivo systems such as animal models and primary tissues.

Already, there is an increasing number of studies aimed at generating specific subtypes of glial cells, mainly astrocytes, to model diseases affected by the corresponding regions of the CNS. Krencil et al. has patterned human pluripotent stem cell-derived astrocytes to rostral-caudal and dorsal-ventral identities by supplementing the morphogens retinoic acid and SHH (Krencil et al., 2011). A recent study by the same group further refined the region-specific astrocyte differentiation from human pluripotent stem cells and demonstrated gene signature specificities and functional diversities of astrocyte subtypes. Xiang et al. developed a fast protocol for generating subtypes of astrocytes by introducing the astrocyte transcriptional factors NFIA and SOX9 into regionally patterned NPCs (Li et al., 2018c). These studies have pioneered the work of generating defined astrocyte subtypes. A recent study generated white-matter and grey-matter astrocytes from both human and mouse iPSCs to model the leukodystrophy disease VWM by coculturing the astrocytes with mouse oligodendrocytes. That study demonstrated the inhibitory effects on oligodendrocyte maturation specifically by white-matter astrocytes (Letenkir et al., 2019). We expect more studies in the near future will generate defined glial cell subtypes and delineate the subtype-specific role of astrocytes in CNS diseases.

With the refinement of glial cell regionality, identity, and functionality, modeling CNS diseases using iPSC-derived glial cells will become increasingly accurate and informative, which will also benefit drug discovery. In addition, iPSC-derived glial cells will benefit glial biology by providing us more information about the species-specific features of human glial cell functions and helping us understand how the evolution of glial cells contributes to the divergence of human brains from that of the other species.

Acknowledgements

We thank Dr. P. Karajanzis for editing the manuscript. This work was supported by the Herbert Horvitz Family, Sidell Kagan Foundation, California Institute for Regenerative Medicine TRAN1-08525, and the National Institute of Aging of the National Institutes of Health R01 AG056305, RF1 AG061794, and R56 AG061171. Research reported in this publication was also supported by the National Cancer Institute of the National Institutes of Health under award number P30CA33572. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

Abud, E.M., et al., 2017. iPSC-derived human microglia-like cells to study neurological diseases. Neuron 94 (2), 278–293 (e9).

Adamczyk, A., et al., 2018. Astrocytic activation generates de novo neuronal potentiation and memory enhancement. Cell 170 (1), 59–71 (e14).

Allen, N.J., Eroglu, C., 2017. Cell biology of astrocyte-synapse interactions. Neuron 96 (3), 697–708.

Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosurg. Rev. 37, 23–39.

Angelova, P.R., et al., 2015. Functional oxygen sensitivity of astrocytes. J. Neurosci. 35 (5), 1178–1186.

Angelova, P.R., et al., 2015. Functional oxygen sensitivity of astrocytes. J. Neurosci. 35 (5), 1178–1186.

Anger, U., et al., 2015. Oxygen-dependent astrocyte activation. J. Clin. Invest. 125 (3), 1095–1104.

Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosurg. Rev. 37, 23–39.

Anger, U., et al., 2015. Oxygen-dependent astrocyte activation. J. Clin. Invest. 125 (3), 1095–1104.

Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosurg. Rev. 37, 23–39.

Angelova, P.R., et al., 2015. Functional oxygen sensitivity of astrocytes. J. Neurosci. 35 (5), 1178–1186.

Anger, U., et al., 2015. Oxygen-dependent astrocyte activation. J. Clin. Invest. 125 (3), 1095–1104.

Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosurg. Rev. 37, 23–39.

Angelova, P.R., et al., 2015. Functional oxygen sensitivity of astrocytes. J. Neurosci. 35 (5), 1178–1186.

Anger, U., et al., 2015. Oxygen-dependent astrocyte activation. J. Clin. Invest. 125 (3), 1095–1104.

Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosurg. Rev. 37, 23–39.

Angelova, P.R., et al., 2015. Functional oxygen sensitivity of astrocytes. J. Neurosci. 35 (5), 1178–1186.

Anger, U., et al., 2015. Oxygen-dependent astrocyte activation. J. Clin. Invest. 125 (3), 1095–1104.

Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosurg. Rev. 37, 23–39.

Angelova, P.R., et al., 2015. Functional oxygen sensitivity of astrocytes. J. Neurosci. 35 (5), 1178–1186.

Anger, U., et al., 2015. Oxygen-dependent astrocyte activation. J. Clin. Invest. 125 (3), 1095–1104.

Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosurg. Rev. 37, 23–39.
Shi, Y., et al., 2017. Induced pluripotent stem cell technology: a decade of progress. Nat. Rev. Drug Discov. 16 (2), 115–130.
Skaper, S.D., et al., 2018. An inflammation-centric view of neurological disease: beyond the neuron. Front. Cell. Neurosci. 12, 72.
Sloan, S.A., et al., 2017. Human astrocyte maturation captured in 3D cerebral cortical spheroids derived from pluripotent stem cells. Neuron 95 (4), 779–790 (e6).
Sofroniew, M.V., 2014. Astrogliosis. Cold Spring Harb. Perspect. Biol. 7 (2), a020420.
Sofroniew, M.V., 2018. Stem cell-derived astrocytes divulge secrets of mutant GFAP. Cell Stem Cell 23 (5), 630–631.
Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol. 119 (1), 7–35.
Sominsky, L., De Luca, S., Spencer, S.J., 2018. Microglia: key players in neurodevelopment and neuronal plasticity. Int. J. Biochem. Cell Biol. 94, 56–60.
Sommer, L., Schachner, M., 1981. Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocyto logical study in the central nervous system. Dev. Biol. 83 (2), 311–327.
Spitzer, S.O., et al., 2019. Oligodendrocyte progenitor cells become regionally diverse and heterogeneous with age. Neuron 101 (3), 459–471 (e5).
Steinman, L., 1996. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 85 (3), 299–302.
Szent, D.B., et al., 2008. Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell 134 (4), 587–598.
Steven, B., et al., 2007. The classical complement cascade mediates CNS synapse elimination. Cell 131 (6), 1164–1178.
Surendran, S., et al., 2003. Canavan disease: a monogenic trait with complex genomic interaction. Mol. Genet. Metab. 80 (1–2), 74–80.
Svoboda, D.S., et al., 2019. Human iPSC-derived microglia assume a primary microglia-like state after transplantation into the neonatal mouse brain. Proc. Natl. Acad. Sci. U. S. A. 116 (50), 25293–25303.
Takahashi, K., et al., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131 (5), 861–872.
Thomas, C.A., et al., 2017. Modeling of TREX1-dependent autoimmune disease using human stem cells highlights L1 accumulation as a source of neuroinflammation. Cell Stem Cell 21 (3), 319–331 (e8).
Trujillo, C.A., et al., 2019. Complex oscillatory waves emerging from cortical organoids model early human brain network development. Cell Stem Cell 25 (4), 558–569 (e7).
Tyzack, G.E., et al., 2017. A neuroprotective astrocyte state is induced by neuronal signal EphB1 but fails in ALS models. Nat. Commun. 8 (1), 1164.
Ullian, E.M., et al., 2001. Control of synapse number by glia. Science 291 (5504), 657–661.
Vainchtein, I.D., et al., 2018. Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. Science 359 (6381), 1269–1273.
Verkman, A.S., 2002. Aquaporin water channels and endothelial cell function. J. Anat. 200 (6), 617–627.
Verney, C., et al., 2010. Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. J. Anat. 217 (4), 436–448.
Wada, T., et al., 2012. Amyotrophic lateral sclerosis model derived from human embryonic stem cells overexpressing mutant superoxide dismutase 1. Stem Cells Transl. Med. 1 (5), 396–402.
Wakselman, S., et al., 2008. Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. J. Neurosci. 28 (32), 8138–8143.
Wang, S., et al., 2013. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. Cell Stem Cell 12 (2), 252–264.
Wenger, D.A., et al., 2000. Krabbe disease: genetic aspects and progress toward therapy. Mol. Genet. Metab. 70 (1), 1–9.
Williams, E.C., et al., 2014. Mutant astrocytes differentiated from Rett syndrome patients-specific iPSCs have adverse effects on wild-type neurons. Hum. Mol. Genet. 23 (11), 2968–2980.
Widrem, M.S., et al., 2014. A competitive advantage by neonatally engrafted human glial progenitors yields mice whose brains are chimeric for human glia. J. Neurosci. 34 (48), 16153–16161.
Widrem, M.S., et al., 2017. Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia. Cell Stem Cell 21 (2), 195–208 (e6).
Yeung, M.S., et al., 2014. Dynamics of oligodendrocyte generation and myelination in the human brain. Cell 159 (4), 766–774.
Zhang, Y., et al., 2016. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. Neuron 89 (1), 37–53.
Zhao, J., et al., 2017. APOE epsilon4/epsilon4 diminishes neurotrophic function of human iPSC-derived astrocytes. Hum. Mol. Genet. 26 (14), 2690–2700.