OPEN ACCESS

University of BRISTOL

Peer reviewed version

Link to published version (if available):
10.1111/bpa.12522

Link to publication record in Explore Bristol Research
PDF-document

University of Bristol - Explore Bristol Research
General rights

Astrocytes in a dish: Using pluripotent stem cells to model neurodegenerative and neurodevelopmental disorders

Lucy A. Crompton[1]*; Oscar Cordero-Llana[2]*; Maeve A. Caldwell[3]

[1] School of Biochemistry, [2]Bristol Medical School, Medical Sciences Bld, University of Bristol, Bristol, BS8 1TD, UK.

[3] Trinity College Institute for Neuroscience, Trinity College Dublin 2, Ireland.

Corresponding author:

Maeve A. Caldwell, Trinity College Institute for Neuroscience, Trinity College Dublin 2, Ireland (Email: Maeve.Caldwell@tcd.ie)

*Authors contributed equally to this work

Abstract

Neuroscience and Neurobiology have historically been neuron biased, yet up to 40% of the cells in the brain are astrocytes. These cells are heterogeneous and regionally diverse but universally essential for brain homeostasis. Astrocytes regulate synaptic transmission as part of the tripartite synapse, provide metabolic and neurotrophic support, recycle neurotransmitters, modulate blood flow and brain blood barrier permeability and are implicated in the mechanisms of neurodegeneration. Using pluripotent stem cells (PSC), it is now possible to study regionalised human astrocytes in a dish and to model their contribution to neurodevelopmental and neurodegenerative disorders. The evidence challenging the traditional neuron-centric view of degeneration within the CNS is reviewed here, with focus on recent findings and disease phenotypes from human PSC-derived astrocytes. In addition we compare current protocols for the generation of regionalised astrocytes and how these can be further refined by our growing knowledge of neurodevelopment. We conclude by proposing a functional and phenotypical characterisation of PSC-derived astrocytic cultures that is critical for reproducible and robust disease modelling.

INTRODUCTION

Astrocytes are the most important neural cell type in the management of homeostatic conditions in the brain (19). They are a type of glia in the central nervous system (CNS), together with oligodendrocytes and microglia. In the adult brain each astrocyte can contact up to a million individual synapses (68). Dendritic spines and presynaptic terminals are intimately wrapped by perisynaptic astrocytic processes forming the tripartite synapse; allowing astrocytes to respond to synaptic activity and in turn regulate synaptic transmission (6, 130).

Recent studies have shown that the cellular composition of the human brain is different to what has been taught for over half a century [for review see (15)]. Historically, the human brain was believed to contain roughly one trillion glial cells and 100 billion neurons yielding a glia to neuron ratio of 10:1. The neurons were thought to be the "talented tenth" due to their ability to communicate via electrical activity and hence perform long distance communication. This exceptional property, not attributable to glia, may in part explain our fascination with neurons and the many in depth studies carried out on neurons in relation to their degeneration. More recently Herculano-Houzel and colleagues have developed and validated an isotropic fractionator method which demonstrated a 1:1 ratio of glia to neurons and hence a glial cell total of 100 billion (74); much lower than previously thought. Whilst astrocytes are known to comprise of up to 40% of all cells within the brain (73), there are regional differences in their numbers; for example, neurons outnumber astrocytes in the cerebellum while the opposite is true in the cortex (73). Such regional differences highlight an important consideration of their potential role in disease pathogenesis.

Astrocytes are a heterogeneous population of cells that have different morphological and physiological characteristics. Traditionally, they are classified into two main subtypes depending on their location within the CNS; protoplasmic astrocytes are mainly found in grey matter and fibrous astrocytes in the white matter (117). However, more recent evidence suggests that this classification is oversimplified and that astrocyte heterogeneity may be an underappreciated topic in neurobiology [for review see (176)], a view supported by a growing number of genomic studies (76, 174). Despite their heterogeneity, it is well established that astrocytes play pan-neural roles in cell to cell communication, metabolic support and also in neurotransmitter recycling (134, 144). In addition to their supportive roles, astrocytes have also been shown to control the formation, function and removal of synapses (53, 131), they also are important in the control of blood flow and critical for the maintenance of the blood brain barrier (BBB) (13, 80, 87, 103).

WHY STUDY ASTROCYTES?

There is an accumulating body of evidence that astrocytes mediate many homeostatic processes which allows us to challenge the traditional neuron-centric view of degeneration within the CNS.

The concept of reactive astrogliosis and its molecular and cellular definition are still incomplete and we are only really starting to understand the multifaceted roles of astrocytes in disease [for review see (18, 133)]. Nevertheless, it is becoming increasingly clear that astrocytic function contributes to the initiation and/or progression of neuronal loss. As reviewed below, multiples studies offer compelling evidence of non-cell autonomous astrocyte dependent mechanisms both in neurodegenerative and neurodevelopmental disorders.

Alzheimer's disease

Alzheimer's disease (AD) —the most common cause of dementia—is characterized by three pathological features: neurofibrillary tangles consisting of intracellular inclusions of hyperphosphorylated tau, extracellular deposits of amyloid beta (Ab) also known as amyloid plaques, and vascular amyloidosis. The exact mechanisms of action are still a topic of debate, however, accumulation of the Ab peptide is believed to result in neuronal dysfunction and eventually cell death (148). Mutations in the amyloid precursor protein gene (APP), APP triplications—as seen in Down Syndrome— or mutations in the APP processing machinery (PSEN-1 & PSEN-2) cause early onset AD [EOAD for review see (54)]. These findings led to the amyloid hypothesis and to a neuronal focus in AD research.

The first evidence that astrocytes play an active role in the pathogenesis was provided by Wyss-Corey and colleagues in 2003. These authors utilized mouse astrocytes to show that they could both take up and degrade Ab (172). This followed on from work that had shown the presence of Ab within astrocytes in the human brain (59, 163) and reactive astrocytosis surrounding amyloid plaques (123). We now know that astrocytes can release a full arsenal of amyloid cleaving enzymes including neprelysin, endothelin converting enzyme-2 and angiotensin-converting enzyme-1 (135). A recent study by Liao et al demonstrated at single cell level that human induced pluripotent stem cells (hiPSC) derived astrocytes can secrete relatively high levels of Ab (101). Astrocytes and not just neurons are also affected by Ab. For example, Ab treatment leads to an increase in intracellular Ca^{21} in rat astrocytes but not neurons. This causes glutathione depletion and astrocytic death followed only indirectly by neuronal loss (2). Ab also causes the release of inflammatory cytokines (IFN-g, IL-1b and IL-6) and astrocyte activation (61). Moreover, astroglyosis can occur in the absence of Ab-plaques (153) and in rat mixed cultures Tau-hyperphosporylation requires the presence of astrocytes (61), indicating that the astrocyte involvement in AD goes beyond amyloid. Disease phenotypes have also been confirmed in human astrocytes from familial and sporadic cases. Jones et al found aberrant morphology and pronounced pathological phenotypes in AD-astrocytes (82), while Kondo et al reported increased endoplasmic reticulum and oxidative stress associated with intracellular Ab in hiPSC derived astrocytes and neurons (89).

Nevertheless, EOAD represents a very small proportion of AD cases and interestingly the genes implicated in Late Onset AD (LOAD) are not specifically neuronal. A GWAS by Harold et al identified three loci associated with AD: ApoE, Clusterin and PIC-ALM (70). PICALM is mainly expressed in endothelial cells from blood vessels in the brain, whereas Clusterin and ApoE (Xu et al, 2006) are typically astrocytic genes. ApoE is a lipid binding protein, highly expressed in the CNS and the best studied risk factor for LOAD. ApoE is expressed predominantly in astrocytes but also in neurons that have undergone injury or stress (23, 50, 110). The human ApoE gene is polymorphic, encoding one of 3 isoforms E2, E3 or E4, which differ by just 2 amino acids, producing substantial isoform-specific properties (111). Unlike genetic mutations that cause autosomal dominant forms of AD, the role of ApoE is more complex (157). Different ApoE alleles modify the risk of developing AD. ApoE3—the most common allele is considered the normal version of ApoE. ApoE4 is associated with an increased risk of AD—5 times for heterozygous and 14 times for homozygous compared to carriers of E3 (43). Interestingly over 65% of AD patients carry a copy of ApoE4. The relationship between ApoE and AD has been attributed to the ability of ApoE to bind Ab for clearance, and its role in transporting lipids for neuronal homeostasis and synaptogenesis (110). The role of ApoE in Ab clearance is attributed in part to the physiology of astrocytes, which internalize and degrade Ab (23). Indeed, astrocytes are associated with Ab plaque degradation and their ability to localize to the plaques requires ApoE (23, 86). Astrocytes from ApoE knockout mice lack this ability while upregulation of ApoE in an Ab over expressing mouse model reversed their AD-like phenotype (23, 88). This suggests that enhancement of astrocyte mediated clearance of Ab is a promising therapeutic target for the treatment of AD, and from work in animal models it is clear that ApoE is fundamental to this process (9, 45, 88).

It is possible that the first alterations leading to AD do not take place in neurons but elsewhere. Indeed, early vasculature changes in endothelial permeability are thought to precede any neurological symptom (30). Interestingly, we know that BBB-function is regulated by the cross-talk between astrocytes and endothelial cells [for review see (1)] and that astrocyte secreted ApoE the biggest genetic risk factor for LOAD is essential to maintain BBB permeability (16). Thus, suggesting that to understand the pathological mechanisms in AD we must look beyond the dying neurons and possibly even beyond astrocytes.

Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease and is characterized by bradykinesia, tremor, rigidity and postural instability. The etiology of this disease is largely unknown, but it involves a complex interaction between various genetic and environmental factors. To date 17 distinctive chromosomal locations or PARK genes (PARK (1–18)) have been identified in familial PD cases. Although only 10% of PD cases are familial, recent GWAS studies have also detected a role for genetic variants in sporadic PD [for review see (58, 96)]. Neuropathologically, there is loss of dopaminergic neurons in the substantia nigra and also Lewy bodies within surviving neurons which are composed of a-synuclein (156). However, the reduced glutathione levels, mitochondrial damage and accumulation of extracellular toxins seen in PD (81) also indicate astrocyte dysfunction.

Mutations and multiplications of the SNCA gene are associated with familial PD and cause early disease onset. Furthermore, the spread of a-synuclein pathology can be used to stage the disease progression (24) and some studies have suggested a prion-like propagation of a-synuclein [for review see (35)]. This occurs not just from neuron to neuron but also from neuron to astrocytes (97),

with the toll like receptor 4 (TLR4) pathway implicated in the uptake of a-synuclein in astrocytes (57, 138). Indeed, there are a plethora of animal based studies that have implicated astrocytes in PD pathogenesis. The gene that has been most extensively studied is DJ-1, which is encoded by Park-7. DJ-1 knockdown in astrocytes results in decreased protection of neurons; for example, impaired protection against the neurotoxins rotenone (121) and 6-OHDA (98). In addition, its mutation alters the expression of proinflammatory mediators, TNF-a and prostaglandin E2 (PGE2), which may provide decreased neuroprotection to surrounding neurons (7) Interestingly, Neumann and colleagues demonstrated that the DJ-1 protein was up-regulated in reactive astrocytes in the human post-mortem Parkinsonian brain with little expression in intra neuronal a-synuclein inclusions (127), suggesting that astrocytes may be involved in neurodegeneration associated with this mutation.

LRRK2 mutations are the most common cause of familial PD (41) and polymorphisms in the LRRK2 locus have been associated with an increased risk of PD (175). Indeed the function of this protein has been intensely studied (42, 49, 64, 115, 169). Pathogenic mutations produce enlarged lysosomes with reduced degradative capacity (72) and are linked to the accumulation of a-synuclein (102).

PINK-1 and Parking have been widely implicated in mitophagy (91). Mutations in these genes lead to autosomal recessive PD. Relating to their role in astrocyte biology PINK1 expression has been shown to increase during stem cell differentiation and brain development and also to affect the development of GFAP positive astrocytes (38). In addition, a deficiency in PINK1 impedes astrocyte proliferation through mitochondrial dysfunction and EGFR downregulation (39). Similarly, glial dysfunction has also been reported in Parkin null mice and midbrain cultures from Parkin mutant mice are resistant to oxidative stress (154, 155).

Amyotrophic lateral sclerosis

Amytrophic lateral sclerosis (ALS) is a devastating and rapidly progressing neurodegenerative condition caused by the degeneration of neurons in the motor cortex, brain stem and spinal cord resulting in muscle paralysis and ultimately motor neuron death (85, 145). Most of ALS cases are sporadic, with around 10% of familial cases [for review see (161)]. ALS is perhaps the neurodegenerative disease where the non-cell autonomous contribution of astrocytes to disease mechanisms has been best established. Early studies showed selective loss of the astrocyte glutamate transporter GLT-1 (143) and markedly reduced glutamate uptake in post-mortem ALS tissue (81). Accordingly, knockout of astrocytic GLT-1 leads to excitotoxicity and neurodegeneration in mice (141). Astrocyte pathology, preceding motor neuron degeneration has also been reported in animal models of SOD1-ALS (48). Multiple in vitro studies have confirmed the importance of astrocytes in ALS. For example, human HB9 positive motorneurons degenerate when co-cultured on primary cortical astrocytes carrying the SOD1^{G93A} mutation. This effect was specific to SOD1^{G93A} astrocytes as over-expression of wild-type SOD1 or co-culture with SOD1^{G93A} mouse embryonic fibroblasts had no effect on motorneuron cell numbers. Non-cell autonomous toxicity occurred even in the absence of glial SOD1 inclusions and was partly mediated by increased release of the pro-inflammatory cytokine PGD2 (48). Using a similar

approach Marchetto et al reported nearly 50% motorneuron loss in co-cultures with primary astrocytes overexpressing mutant SOD1^{G73R}. Interestingly, the effect was motorneuron specific as other neuronal cell types in the dish—such as GABA[1] neurons were not affected (113). More recent studies have extended the involvement of astrocytes beyond SOD1-ALS. Almad et al found increased Connexin 43 expression, astrocyte coupling and intracellular calcium not only in SOD1^{G93A} astrocytes but also in C9ORF72 expansion carriers and sporadic cases of ALS (4); a tan- talizing finding that would make Connexin 43 and astrocytes a cen- tral node in the pathophysiological mechanisms for multiple ALS subtypes.

From the TDP43-ALS side we know that mutations in the TARDBP gene—which encodes TDP-43—affect the survival of hiPSC-derived neurons and astrocytes (11), with increased cytoplasmic levels of TDP-43 leading to over twofold increase in the risk of cell death in TARDBP^{M337V} astrocytes (149). This confirms that the nuclear-cytoplasmic ratio of TDP-43 must be finely tuned not just in neurons but also in astrocytes. Interestingly, TARDBP^{M337V} astrocytes had no direct effect on the survival of wild-type motorneurons (149), which would suggest that—unlike the case of SOD1—the ALS mechanisms mediated by TDP-43 appear to be largely cell autonomous. Furthermore, mutations in the MAPT locus associated with Fronto-Temporal Dementia lead to astrocyte pathology in vitro—with hypertrophy, increased 4R-Tau expression and exacerbated sensitivity to oxidative damage in human MAPT^{N279K} astrocytes (69). This is important because ALS and FTD are considered part of the same disease continuum and together, these data implicate astrocytes all the way from pure SOD1-ALS to the MAPT associated FTD spectrum.

Huntington's disease

Huntington disease (HD) is caused by a trinucleotide expansion in the Huntingtin gene located in chromosome 4. Huntingtin (HTT) is ubiquitously expressed and mutant HTT has been shown to accumulate both in human neurons and astrocytes (55). Multiple mechanisms have been proposed to explain the preferential loss of striatal medium spiny neurons (MSN) in HD. These include glutamate excitotoxicity, oxidative stress, mitochondrial dysfunction and loss of neurotrophic factor support—for review see (63). That astrocytes are an integral part of these, is becoming increasingly clear. For example, transgenic mice, expressing mutant HTT under the GFAP promoter show age-dependent behavioral phenotypes, reduced EAAT1 expression and reduced striatal glutamate uptake—albeit no MSN loss (25). In the R6/2 mouse model of HD, striatal astrocytes have lower K[1] conductance and reduced Kir4.1 potassium channel expression and of these 20% display mHTT nuclear inclusions. Lower K[1] conductance in astrocytes leads to higher extracellular K[1] concentration and increased depolarization and excitability of MSN (164). This occurs before any evident signs of gliosis. Remarkably, restoring astrocytic Kir4.1 levels by viral delivery attenuated the disease phenotypes in R6/2 mice—improv- ing motor phenotypes and prolonging life-span (164). From another angle, loss of BDNF expression and neurotrophic support has also been linked to HD (179). BDNF is produced by both neurons and astrocytes and, at least in mice, expression of mHTT has been shown to impair astrocytic BDNF secretion (77). Furthermore, expression of mHTT in mouse astrocytes causes loss of wild-type

neurons in co-cultures in vitro (152). Whether these findings hold true for human astrocytes and neurons remains to be investigated. To our knowledge only one study has explored disease phenotypes in human astrocytes derived from HD hiPSC lines. Juopperi et al, reported increased vacuolization in the cytoplasm of astrocytes expressing 109CAG-mHTT—compared to control 28CAG-HTT cells (83). However, the significance of this finding or how this affects astrocytic function in HD is unclear.

Down's syndrome

Down Syndrome (DS) is a neurodevelopmental disorder caused by trisomy of chromosome 21. Besides being the most common cause of intellectual disability, DS is also associated with a much higher risk of developing early onset AD. The APP locus is located in the chromosome 21 (21q21.3). Therefore, trisomy causes continuous overproduction of amyloid, leading to development of neurofibrillary tangles and amyloid plaque pathology by the age of 40. By the age of 60, more than 60% of DS sufferers will develop dementia (171).

In addition to the traditional difficulty of studying developing human neural cells, modeling full chromosome 21 trisomy is not possible in mice, which has hindered DS research. This makes hiPSC-technology particularly attractive for the study of DS. Moreover, as there is a small degree of mosaicism in DS fibroblasts, it is possible to generate euploid and aneuploid hiPSCs clones from the same affected individual (168). It is also possible to generate euploid subclones from otherwise karyotypically stable trisomic hiPSC lines (109). This means that obtaining isogenic control lines—critical for a multilocus disorder such as DS—is relatively straightforward. Despite early studies implicating glial pathology—including reactive astrogliosis and upregulation of S100b and IL-1 (65) research in DS has focused on neuronal dysfunction. Shi et al found intracellular aggregation of Ab42 and tau hyperphosphorylation associated with increased cell death in cortical neurons derived from 21-trisomic hiPSCs (151), while Weick et al reported increased oxidative stress vulnerability and reduced ability to form functional synapses in these neurons (168). A study by Briggs et al confirmed the sensitivity of these neurons to oxidative stress but interestingly also found a two-fold bias in neural differentiation towards glial lineages—given by the number of GFAP[1] cells—as well as increased expression of other astroglial genes including EAAT1, S100b and NF1A (27). Of note, the S100b genomic locus 21q22 is also triplicated in DS and it is known that S100b overexpression can cause gliosis (119) and stimulate iNOS expression (78). In an elegant study, Chen et al confirmed a higher expression of S100b and iNOS in hiPSC-derived DS-astrocytes compared to isogenic disomic controls. These astrocytes showed faster proliferation rates, increased production of reactive oxygen species and NO, together with reduced ApoE, BDNF, Nrf2, Tsp-1 and Tsp-2 expression, suggesting a shift from a neuroprotective/neurotrophic profile towards a more pro-inflammatory/neurotoxic phenotype. Accordingly, S100b knockdown or pharmacological treatment with the anti-inflammatory and anti-oxidant antibiotic minocycline were able to partially revert these phenotypes. Together, these data indicate that early glial alterations could underlie the developmental effects seeing in DS.

Other neurodevelopmental disorders

Rett syndrome is a rare neurodevelopmental disorder caused by mutations in the methyl-CpG-binding protein 2 (MeCP2) gene located in the X-chromosome. A DNA binding protein, MeCP2 has been involved in transcriptional activation, transcriptional repression, retrotransposon silencing and chromatin remodeling [for review see (107)]. hiPSC are particularly well suited for Rett-syndrome modeling: X-chromosome reactivation during hiPSCs reprogramming followed by random inactivation during somatic cell differentiation means that it is possible to generate quasi-isogenic lines from heterozygous female carriers. Furthermore, because MeCP2 functions are not restricted to neurons, it is important to profile MeCP2 mutations in all neural cell types. Indeed, non-cell autonomous effects of MeCP2 mutations on dendritic morphology have been reported in mouse astrocytes (10). Similarly, human neurons co-cultured with hiPSC-derived astrocytes carrying MeCP2 mutations are morphologically abnormal—showing reduced somas, neurite length and branching (170). Altered microtubule dynamics, increased non-directional movement of lysosomal vesicles has also been reported in hiPSC-derived MeCP2R294X astrocytes (47). These phenotypes could be recovered with Epothilone D, a microtubule stabilizing drug (47), highlighting the potential of hiPSC-astrocyte modeling to uncover novel pharmacological targets for this condition.

Alexander's disease is an extremely rare neurodevelopmental condition, with only around 500 cases described to date. Symptoms typically appear before the age of two and include megalencephaly, seizures, stiffness and intellectual disability. Histopathologically, the disease is characterized by the presence of Rosenthal fibers in astrocytes—intracellular aggregates of GFAP, HSP-27 and ab-crystallin [for review see (147)]. Astrocytes have been the focus of Alexander's disease research since the discovery of causative dominant mutations in the GFAP gene (26), which made this condition the prototypical astrocytic genetic disorder. Clearly, modeling Alexander's Disease in hiPSC derived astrocytes has great potential. Especially since human astrocytes seem to show strong disease phenotypes, including Rosenthal-like GFAP aggregates, altered gene expression profile an increased production of IL-5, IL-6 and TNF-a (90).

THE POWER OF HUMAN IPS CELLS TO MODEL NEURODEGENERATIVE DISEASE AND NEURODEVELOPMENTAL DISORDERS

To date, there have been many approaches to studying CNS diseases, which include classical cell lines such as HEK293, SHY5Y cells and primary cultures from rodents. Another avenue of research has utilised whole animal models, in particular rodents overexpressing proteins that play key roles in the disease. This research has been undeniably informative but has intrinsic flaws. Most often the protein in question is present at much higher levels than would normally exist in the human brain. Furthermore, any non-human model of human disease is fundamentally limited; rodents do not develop AD, PD or DS, and this highlights crucial inter species differences. For these reasons it has been difficult to translate findings into the clinic. Most strikingly various drugs that

showed promise in animal models have failed human trials (32). Therefore, there is a requirement for a model where proteins involved in neurodegenerative and neurodevelopmental disorders are studied at native levels and in human cells, thus more closely recreating the environment of the human brain. iPS cell technology can generate "vulnerable" neural subtypes from patients with the disease offering an unprecedented insight into cell type specific pathology, something that cell lines and primary cultures cannot. In addition, as discussed above, neuropathological mechanisms involve interactions between neurons and glia which cannot be modelled by generating neurons alone. Furthermore, there is a long list of interspecies differences between rodent and human astrocytes which underlies the need for authentic human astrocytes for appropriate disease modeling [for review see (34)]: (i) Human astrocytes are more complex than their rodent counterparts (128). (ii) Human astrocytes display twice as many processes as their rodent counterparts (176). (iii) Two main astrocytes—proteoplasmic and fibrous-types are found in the mouse brain while two additional subtypes have been identified in primates (40). (iv) This structural complexity is also reflected in functional properties: human astrocytes propagate calcium waves more rapidly than rodent counterparts and have a greater response to glutamate (158, 177). (v) There are striking differences in their transcriptomic profiles, with over 600 genes expressed in human astrocytes that are not active in rodent astrocytes (176). The potential of iPSC-derived astrocytes for disease modeling is illustrated in Table 2, which summarizes pathological mechanisms and disease phenotypes uncovered in these cells.

ASTROCYTE DIFFERENTIATION— TAKING CUES FROM THE EMBRYO

Due to the significance of astrocytes in the CNS, their efficient generation from human pluripotent stem cells (hPSCs), is essential for disease modelling. To achieve the differentiation of hPSCs into the vast array of cell types of the CNS, researchers have looked to embryonic development to inform us of the cues required (105). In the embryo, the processes of cellular differentiation and regional patterning are tightly regulated and their correct specification is key to generate a correctly functioning CNS. These must be recapitulated in vitro with hPSCs to accurately generate the specific functional cell populations. There are certain crucial requirements of hPSC differentiation protocols: (i) Acquisition of neural progenitor fate and loss of pluripotency; (ii) Regional patterning of the progenitors, relating to the positional identity of the resulting mature cell population in the CNS; (iii) Successful generation the type of cell required; (iv) Characterization of the resulting cells to confirm true identity and function. Here we will discuss how these principles are being met in relation to the generation of astrocytes from hPSCs.

The acquisition of neural identity

The first event of cellular specialization in the embryo is differentiation of the three embryonic germ layers, mesoderm, endoderm and ectoderm (106). The ectoderm gives rise to the neuroepithelium, which is composed of the neural progenitors that give rise to all specialized cells of the CNS. These early developmental events are recapitulated when differentiating hPSCs (114, 166). Many neural differentiation protocols utilize an Embryoid Body (EB) stage in the protocol—the EB is the established method to recapitulate and model early embryonic development using mouse or human Embryonic Stem Cells (ESCs) (114, 166). Using mechanical or enzymatic dissociation ESCs are seeded in 3D culture and form non-adherent spheres. Removing the factors required for maintaining pluripotency, LIF for mouse and FGF signaling for human, results in spontaneous differentiation of the three embryonic germ layers and subsequently their more specialized derivatives (166). Differentiation of hPSCs can also be achieved as a monolayer of adherent cells, and there has been somewhat of a division in the field between EB-based vs. monolayer differentiation (Table 1). Some would argue that differentiation of hPSCs as a monolayer may be easier to "program" as by its nature EB based differentiation recapitulates many of the endogenous signaling pathways of the early embryo. For directed differentiation of a single desired cell type these signaling pathways may yield undesirable effects generating unwanted cell types. However, EB based differentiation is still used very successfully by many labs as a basis for hPSC differentiation (Table 1). The field has also seen the advent of chemically defined media where every component is known and characterized, essentially providing a 'blank canvas' for unbiased cell differentiation that can be directed towards a desired fate (165).

In the early embryo BMP and Nodal signaling inhibit neural differentiation (106). Nodal and BMP signal transduction occurs via SMAD proteins, and inhibition of SMADs has been adopted by the stem cell field to enable differentiation of hPSCs into neural lineages (166, 167). Chambers and colleagues first published what they termed "dual SMAD inhibition," using the small molecule inhibitor SB431542 to block Nodal signaling, and a recombinant form of the embryonically expressed protein Noggin to inhibit BMP signaling, generating neural progenitors from hPSCs with high efficiency (33). More recent protocols replace Noggin by small molecule inhibitors of BMP signaling, most commonly dorsomorphin or LDN193189 (94, 146, 178). In fact small molecules have been uni- versally adopted for the directed differentiation of hPSCs as they demonstrate potent efficacy and are more cost effective compared to recombinant proteins (130). Dual SMAD inhibition is now the "gold standard" for the generation of neural progenitors from hPSCs and has been used to produce a diverse range of both neu- rons and astrocytes (105).

Once neural progenitors are specified, it is advantageous that these are expandable in culture. In vivo, embryonic neural progenitors are required to be highly proliferative to enable generation of the large numbers of neural cells within the adult CNS. Similarly in vitro this means that only a small starting number of hPSCs are required to generate large numbers of mature cells. In the developing embryonic CNS progenitor populations proliferate in response to first FGF and later to EGF signaling (29, 60). Therefore, the addition of these mitogens to hPSC derived neural progenitors to drive expansion has become commonplace (46, 90, 93, 105). However, some hPSC differentiation protocols do not require the addition of mitogens for proliferation of the neural progenitors, and it is presumed that in these systems the progenitors produce autocrine signals to induce proliferation (33, 94). However, addition of exogenous mitogens can enable hPSC derived neural progenitors to proliferate in vitro culture for longer periods (46, 90, 93, 105) (Table 1).

Table 1. Astrocyte differentiation from human induced pluripotent stem cells.

Brain region	Patterning factors	Astrogliogenic factors	Initial differentiation	Method of differentiation	Timings required from hPSC/yield	Markers determined	References
Various	FGF8A, RA SHH	None	EB	EB are plated onto culture surface & neural rosettes undergo physical selection & expanded as nonadherent spheres	180d >90% GFAP, S100b	S100b/GFAP/ Aldh1L1	Krencik et al, 2011
N/A	None	BMP2 BMP4 LIF	EB	EB cultured in chemically defined media. At day 8 neural progenitors then plated onto culture surface and expanded (↑EGF↑FGF2↓Heparin).	67d >95% GFAP	S100b/GFAP/ AQP4/EAAT1	Gupta et al, 2011 (67)
Ventral spinal cord	RA Purmorphamine	LIF CNTF	Monolayer	Dual SMAD inhibition on monolayer hPSCs. Neural progenitors expanded as nonadherent neurospheres (↑EGF↑LIF followed by ↑EGF↑FGF).	>56d >90% S100b, GFAP	Vimentin/ S100b/GFAP	Serio et al, 2013 (149)
Ventral spinal cord	RA SHH	CNTF FBS	Monolayer	Dual SMAD inhibition on monolayer hPSCs. Neural progenitors expanded in commercial media	80–100d rv100% S100b	S100b/GFAP CX43/Aldolase-C/ EAAT2	Roybon et al, 2013 (146)
N/A	None	BMP2 LIF Trichostatin A Aza-cytidine	Monolayer	Neural progenitors differentiated from hPSCs as an adherent monolayer.	52d Majority GFAP (not quantified)	GFAP/S100b/ EAAT1/ALDH1L1	Majumder et al, 2013 (112)
N/A	None	BMP4	EB ➔ Monolayer	EB are plated onto culture surface & neural rosettes undergo physical selection & expanded as nonadherent spheres	42d 60% S100b	GFAP. Vimentin, S100b	Chen et al, 2014 (36)
N/A	CHIR99021 SHH	LIF FBS	EB ➔ Monolayer	Dual SMAD inhibition during EB formation. Neural rosettes isolated as neurospheres then plated onto culture surface and expanded (↑EGF↑FGF2↓LIF)	80d 100%	Vimentin/S100b/ GFAP/AQP4/EAAT2	Palm et al, 2015 (129)
N/A	None	None	EB ➔ Monolayer	Dual SMAD inhibition during EB formation. Neural progenitors then plated onto culture surface and expanded (↑EGF↑FGF2↓Heparin)	180d rv90% GFAP, S100b	GFAP/S100b	Kondo et al, 2016 (90)

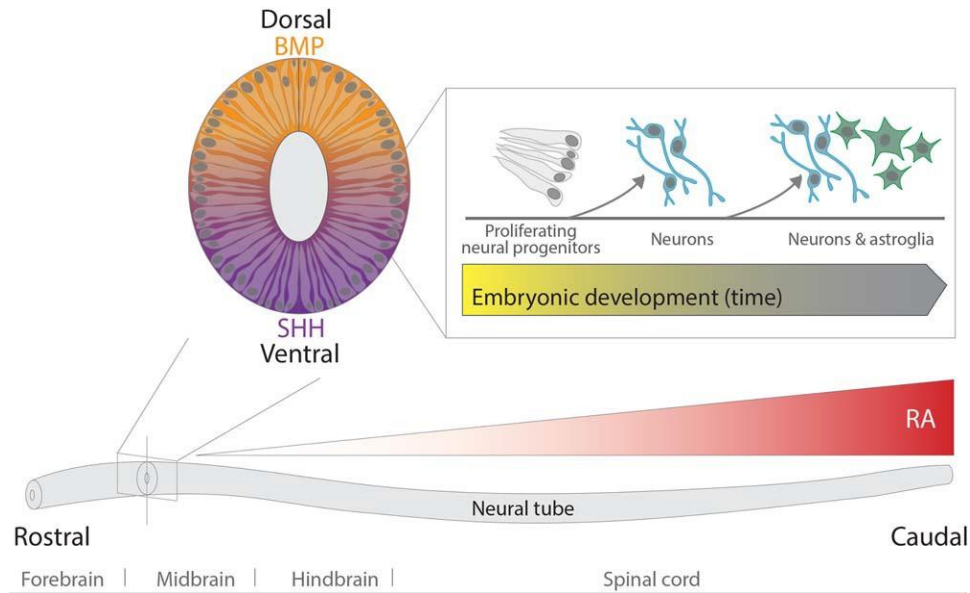Abbreviations: d ⁼ days; RA ⁼ retinoic acid.

Figure 1. Differentiation and patterning in the developing CNS. The CNS develops from the neural tube which is made up of neural progenitor cells. In the early embryo neural progenitors can only generate neurons and only later become capable of differentiating into glial cells, including astrocytes. This temporal fate switch is recapitulated in vitro in both ex vivo neural progenitor cultures and also in the differentiation of hPSCs. Cells within the CNS acquire regional identity according to gradients of morphogens expressed across the neural tube. The rostrocaudal axis is set up by a gradient of retinoic acid (RA), which is highest where the future spinal cord will form. The dorsoventral axis is set up by opposing gradients of ventral SHH signaling and dorsal BMP signaling. These axes facilitate the correct spatial patterning of the developing CNS.

Astrogliogenesis

Astrogliogenesis refers to the generation of glial cells, in particular astrocytes, either in vivo or in vitro. In the embryonic brain neurons and astrocytes differentiate from the same neural progenitor pool. However, there is a distinct temporal shift from neuronal to glial fate acquisition (120) (Figure 1); initially neural progenitors are monopotent, generating neurons and only later become bipotent, differentiating into both neurons and glial cells, including astrocytes (Figure 1). This temporal control is also recapitulated in vitro, in neural progenitor cultures ex vivo and in hPSC differentiation (93, 105), suggesting that fundamental intrinsic mechanisms control the switch. The identification of a single specific cue that induces the neuron-to-glial fate switch remains elusive, but research has uncovered a number of essential signaling pathways (120). In relation to hPSC differentiation, astrocytes will differentiate from hPSC derived neural progenitors by 'default' after elongated peri- ods in culture (90, 93, 105). For a cell population highly enriched for astrocyte culture periods of over 180 days are required (90, 93). Therefore, factors to promote the glial fate switch and astrocyte differentiation are frequently added to protocols to reduce culture times and increase the yield of astrocytes. A classic approach for generating astrocytes from ex vivo neural progenitors was to add animal serum to the culture media (137). This approach has also been used successfully with hPSC derived neural progenitors (100, 129, 146). However, an undefined component like animal serum could lead to lack of reproducibility and other unwanted effects on the neural cells, particularly when using hPSC derived neural cells for disease modelling. Therefore, researchers have looked to astrogliogenesis in the embryo for candidate molecules and signaling pathways that could be utilized to promote the generation of astrocytes (105).

In particular the Interleukin-6 (IL-6) family of cytokines including Ciliary Neurotrophic Factor (CNTF), Leukemia Inhibitory Factor (LIF) and Cardiotrophin (CT-1) promote the generation of astrocytes via induction of downstream JAK-STAT signaling (21, 71). Activated JAK (Janus Kinases) tyrosine kinases in turn activate Signal Transducers and Activators of Transcription (STAT) proteins which function as transcriptional activators at astrocyte specific loci, such as GFAP and S100b (21, 71). LIF and CNTF are frequently used in the generation of astrocytes from hPSCs (149) (Table 1). One study by Serio and colleagues generated astrocytes from hiPSCs as part of an in vitro model of CNS proteinopathy. They achieved over 91% GFAP positive cells from multiple hiPSC lines using an approach of extended expansion of hiPSC derived neural progenitors in the presence of EGF and LIF, followed by terminal astrocytic differentiation with CNTF. The period of expansion required was much reduced compared to naïve generation (93, 149).

Clearly the IL-6 family of cytokines and subsequent JAK-STAT signaling is crucial to initiation of astro gliogenesis. However high levels of JAK-STAT signaling alone cannot override the program for the initial generation of only neurons (21, 71, 75), indicating that the neuron to astrocyte switch is controlled by multiple mechanisms. BMP and NOTCH signaling also synergistically promote astrocyte differentiation, but only in the presence of active IL6/JAK-STATs (62, 75, 84, 124, 125). BMP signaling is context specific; in early CNS development it promotes the generation of neurons and only in the later gliogenic period do BMPs promote the differentiation of astrocytes (99, 108, 159). BMP downstream signal transducers, the SMAD proteins interact with the CREB-

Table 2. Disease Phenotypes in hiPSC-derived astrocytes.

Disease	Gene	Findings		References
Alexander's Disease	GFAP	GFAP aggregates (Rosenthal like fibers) Compromised cell adhesion	"Il-5 "Il-6 "TNF-∕ "mTOR signaling	Kondo et al, 2016 (90)
ALS	TARDBP^{M337V}	"Cell toxicity #Astrocyte survival		Barmada et al, 2014 (11)
	TARDBP^{M337V}	"Cytoplasmic TDP43 levels # Astrocyte survival	Cell autonomous effect	Serio et al, 2013 (149)
	SOD1 C90RF72 Sporadic	"CRX43 expression "Astrocyte Coupling?		Almad et al, 2016 (4)
Alzheimer's	APP ^{V717I}	Single-Cell secretion profiling	Significant astrocytic Ab secretion	Liao et al, 2016 (101)
Disease	PSEN^{M146L} ApoE4$^{1/1}$	Aberrant morphology Astrocytic atrophy #S100b #EEAT1 #GS	Conditioned medium "CCL5, "MIP-1b #IL-8 #MCP-1	Jones et al, 2017 (82)
Down's Syndrome	21 Trisomy	" S100b astroglia differentiation "Proliferation #Nrf2 #BDNF #ApoE " iNOS "ROS	Conditioned medium #Neurogenesis #Neurite outgrowth #Neuron maturation #Synapse formation	Chen et al, 2014 (36)
FTD	MAPT^{N279K}	Enlarged astrocytes "Oxidative damage sensitivity "Protein degradation	"S100b "ANXA2 "NPY "MAOB	Hallman et al, 2017 (69)
HD	HTT	Astrocyte Vacuolization		Juopperi et al, 2012 (83)
Rett Syndrome	MeCP2^{R294*}	" Microtubule growth " Microtubule transport " Non-directional lysosomal movement	#Acetylated ∕-tubulin	Delépine et al, 2016 (47)
	MeCP2^{V247*} MeCP2^{R294*} MeCP2^{R306C}	Co-culture #Neurite length #Neurite number #EPSCs	Conditioned medium #Neurite length #Neurite number #EPSCs	Williams et al, 2014 (170)
SMA	SMN1	"Catalase #ROS levels	"# Respiration "#MnSOD, Nrf2	Patitucci et al, 2016 (132)
	SMN1	"GFAP #Astrocytic processes "Resting Ca^{21} levels	#GDNF #ATP-induced Ca^{21} response	McGivern et al, 2013 (116)
X-linked ALD	ABCD1		"Il-6	Baarine et al, 2015(8)

Abbreviations: ALS 5 amyotrophic lateral sclerosis; FTD 5 frontotemporal dementia; SMA 5 spinal muscle atrophy; ALD 5 adrenoleukodystrophy.

binding protein (CBP)/p300 transcriptional activator complex, activating genes containing a CBP binding site in their promoter region. Nakashima et al, demonstrated that CBP/p300 further com- plexes with the STAT protein STAT3, downstream of the IL-6 cytokine pathway, resulting in direct activation of astrocyte genes including GFAP (124). Accordingly the pro-astrocytic properties of animal sera are attributed to BMP2 and BMP4 (79). In terms of hPSC differentiation, BMPs are often used together with the IL-6 family cytokines to synergistically enhance astrocyte generation as they do in the developing CNS (67). Gupta and colleagues demon- strated

that co-application of BMP4 and LIF significantly increased the generation of functional astrocytes from human ESCs (67). In this study, a combination of BMP4 and LIF generated over 95% GFAP positive cells in 67 days. Similar approaches have now been applied to hiPSCs for the highly efficient generation of astrocytes for disease modeling (16).

Notch signaling is another context specific regulator of astrogliogenesis; during neurogenesis Notch signaling acts to maintain the neural progenitor pool. Like BMP signaling, Notch is only able to induce astrogliogenesis if JAK-STAT signaling is active (62, 84). Notch signaling leads to transcription of the Hairy Enhancer of Split (Hes) gene and the Hes protein then binds to active JAK and STAT proteins and forms complexes which also act as transcriptional activators (62, 84). Notch activation increases expression of GFAP as the GFAP promoter contains Notch responsive elements (62).

From our knowledge of the role of Notch signaling in astro gliogenesis it is logical that the Notch pathway could be exploited for astrocyte generation from hPSCs (37, 126). Accordingly the inhibition of Notch signaling is commonly used to increase the efficiency of neuronal differentiation from hPSCs via use of the g-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenyl-glycine

t-butyl ester (DAPT) (20, 22, 94). However, the multiple roles of Notch signaling in the development of the CNS make this a difficult pathway to target. One study by Emdad et al, attempted to differentiate astrocytes from hPSCs by stimulating Notch signaling in combination with CNTF but saw no pro-astrocytic effect on gene expression (51). However, this is a single study and this result is in direct disagreement with work on ex vivo rat embryonic neural precursors, where Notch activation in the presence of CNTF increased generation of astrocytes compared to CNTF alone (122). This may be down to the different methods of Notch activation used in the two studies, species differences, or other differences in culture conditions, but the targeting of Notch signaling for the generation of human astrocytes does warrant more investigation.

As with all cellular differentiation, the generation of astrocytes is under the restraint of epigenetic remodelling. The temporal fate shift of neural progenitors results from changes at neuronal vs glial gene loci, causing downregulation of pro-neuronal genes and upregulation of astrocyte genes (21, 56, 71, 75). This is ultimately achieved by epigenetic changes in parallel and in conjunction with the discussed signaling pathways. DNA methylation blocks the binding of STAT proteins and the activation of astrocytic genes during early CNS development; conversely global inhibition of DNA methylation enables premature astrocyte differentiation via JAK-STAT signaling activating the demethylated and therefore permissive astrocytic gene loci (21, 56, 71, 75). Notch signaling also acts directly on the epigenetic status of multiple astrocytic gene promoters; Namihira and colleagues demonstrated that active Notch signaling induces demethylation of the promoter via binding of the NFIA transcription factor (126). NFIA acts by protecting the promoter from methylation by DNA Methyltransferase 1 (DNMT1) and the resulting demethylation enables transcriptional activators, such as the STATs, to bind to these promoters and induce gene expression (126). For this reason activation of Notch signaling is also a prerequisite for the ability of JAK-STAT proteins to induce astrocytic gene expression, but it cannot promote astrocyte differentiation without JAK-STAT signaling (37, 126).

An alternative approach to generate astrocytes would be to directly modulate the epigenetic status of hPSC or the hPSC derived neural progenitors. Inducing a "glial-permissive" epigenetic landscape could prematurely induce astrogliogenesis, and further reduce required culture time. One study by Majumber et al used the DNA methyltransferase inhibitor Aza cytidine, and the histone deacetylase inhibitor Trichostatin-A, in combination with BMP2 and LIF on hPSC derived neural progenitors (112). They reported reduced methylation and increased acetylation of astrocytic gene promoters, including that of GFAP and S100b compared to untreated controls, and correspondingly the premature appearance of astrocytes (112). In support of this pharmacological epigenetic modification has shown comparable effects on rodent neural progenitors in vivo and in vitro (150, 160). Off target effects prevent these approaches from being adopted more widely. Indeed, such treatments lead to widespread cell death (112) and may have an impact on any disease phenotype.

It is clear that as in the developing brain astrocytes differentiate in vitro from hPSC derived neural progenitors following a default developmental timeline. Studies that have achieved high astrocyte yields without the addition of extrinsic factors tend to require much longer time periods in culture (90, 93) (Table 1). In vivo there is a requirement for the presence of differentiating neurons to induce

astrocyte differentiation, as neurons are the source of Notch ligands (37, 126) and CT-1—a member of the IL-6 family—which induces JAK-STAT activation (12). This may explain in part why neurogenesis and astrogliogenesis happen in a sequential manner. Therefore most popular strategies to decrease differentiation time in vitro and increase efficiency of astrocyte differentiation are to target these pathways required in the embryo, to an extent substituting for the required presence of neurons (Table 1 and references within).

Regional identity and astrocyte function

Another requirement for cellular specialization within the CNS is acquisition of regional identity. In the embryo, the neuroepithelium starts as a plate-like structure which then folds and fuses to forming the neural tube. The neural tube develops both rostrocaudal and dorsoventral axes, which provide the outline for the spatial patterning required to generate the different regions of the mature CNS (Figure 1). The axes are set up by gradients of specific morphogens. The rostrocaudal axis is set up by a gradient of retinoid signaling via Retinoic Acid (RA), highest in the caudal extreme where the future spinal cord will form (162) (Figure 1). The dorsoventral axis is regulated by Sonic Hedgehog (SHH), expressed ventrally along the length of the neural tube, and BMP signaling, highest at the dorsal side of the neural tube (162) (Figure 1). Both neurons and astrocytes possess regional specific identity and consequently express region specific markers (31, 93). Regional identity is key to function, phenotype and even the morphology of neural cells. The importance of regional identity to neuronal function is historically well documented, but only more recently it has been recognized that the regionalization of astrocytes is similarly integral to their function (31, 44, 93). This relates to their effective support of the specific neuronal populations within that region (13, 31, 44, 92, 93, 95, 136, 176). For example, astrocytes in the cortex cannot provide the trophic support required by midbrain neurons (44). Therefore, when generating astrocytes from hPSCs for in vitro study or transplantation, we must accurately assign this regional identity. Researchers are now able to generate a vast array of regionally patterned neuronal cell types, for example, cortical glutamatergic, ventral forebrain cholinergic, ventral midbrain dopaminergic and cholinergic motor neurons (48, 93, 94, 105, 151). The same principles are now being combined with our knowledge of astrocyte development, to generate regionally patterned astrocytes (see Table 2).

This is achieved by treatment with developmental molecules, in particular the aforementioned, RA, BMPs and SHH, thus recapitulating signaling in the developing CNS (105) (Table 1). A seminal study by Krencik et al, demonstrated that regionalized astrocytes and neurons could be generated from the same pool of hPSC derived neural progenitors. Krencik and colleagues demonstrated a universal protocol that could be 'fine-tuned' by the addition of such morphogens to the hPSC derived neural progenitor pool (93). The progenitors retained this patterning even after the 180 day expansion period required for the appearance of astrocytes (93). These astrocytes correctly recapitulated regional identities spanning the rostrocaudal and dorsoventral axes of the CNS (93). For example, addition of SHH resulted in expression of the ventral forebrain transcription factor NKX2.1 in S100b[1] astrocytes, whereas addition of RA resulted in expression of caudal transcription factor HOXB4 (93). Roybon et al used a similar approach to successfully generate ventral spinal cord astrocytes by combined addition of SHH and

RA (146). These findings agree with the regional astrocytic phenotypes seen in vivo and highlight how crucial generating regional-specific astrocytes is for accurate disease modeling (31, 44, 176).

STANDARDIZATION AND FUNCTIONAL CHARACTERIZATION

A major issue when generating any cell type from hPSCs is suitable phenotypic and functional characterization. It is absolutely crucial that the stem cell field adopts a standardized and multifaceted strategy to characterize hPSC derived cells, because this will enable direct comparison of derivation methods and the resulting cells.

One cannot model a disease without the bona fide cell type that the specific disease affects in vivo, and clearly these cells must be functional if we are to determine how function is affected in disease. In terms of neural differentiation, we are starting to see a set of standard characterization methods emerge. Here we will discuss those required for characterization of hPSC derived astrocytes, and how this may impact the field.

As discussed astrocytes are a very heterogeneous cell type and this diversity extends to their morphology and expression of what are classed as "typical" astrocyte proteins such as GFAP or S100b (176). If we just compare the morphology of the two crude astrocytic subclasses, they are remarkably different. When we think of the classical "star-like" astrocytes these are in fact fibrous astrocytes. In comparison protoplasmic astrocytes have a more irregular, 'bushy' appearance, extending long sheet-like processes (117, 118, 128). The morphology of astrocytes also changes depending on their proliferative or reactive status (14, 128). Furthermore, astrocytes differentially express GFAP, which is traditionally considered and still used by many as the first line astrocytic marker. Protoplasmic astrocytes generally express GFAP at much lower levels than fibrous astrocytes and high levels of GFAP are also a feature of reactive astrocytes (118, 128). This heterogeneity therefore makes the identification of astrocytes both in vivo and in vitro very difficult and this also translates identifying astrocytes generated from hPSCs. What is the best strategy to confirm and evaluate the generation of astrocytes form hPSCs?

A seminal study by Cahoy et al has contributed a range of novel astrocyte specific markers. Transcriptome analysis compared astrocytes, neurons and oligodendrocytes from early vs. late postnatal mouse forebrain (28). Two markers in particular, ALDH1L1 and Aldolase-C were shown to be astrocyte specific (28). Antibodies for detection of these markers are commercially available and have been used for the identification of astrocytes, including hPSC derived astrocytes (28, 52, 118) (Table 1). In addition the same study confirmed astrocyte specific expression of Aquaporin-4 (AQP4), EAAT1 (SLC1A3 or GLAST) and GFAP, along with S100b, which was used to identify astrocytes and oligodendrocytes (28). Therefore, we now have a library of confirmed astrocytic markers that can be used in combination with cell morphology to better identify true astrocytes and distinguish them from other glial cell types. In addition these astrocytic markers can be used in combination with classical regional CNS markers to establish their regional identity (93).

However, confirmation of marker expression alone provides no information about astrocyte function. Particularly for disease modeling, derivation of astrocytes from hPSCs requires functional validation. This is somewhat difficult because we are still learning so much about astrocytes in vivo and their many functions in the brain (176). One of the main roles of astrocytes in vivo is to respond appropriately to neuronal signals including neurotransmitters such as glutamate or ATP, calcium ions and electrical signals (13). Astrocytic response can be measured by patch clamping individual astrocytes. However, more commonly hPSC-derived astrocytes are assessed by calcium imaging. These stimuli result in changes in intracellular calcium stores, fluorescent calcium dyes can be visualized and quantified in many cells simultaneously (92). This high throughout approach is advantageous when evaluating a large population of astrocytes derived from hPSCs, and yields a more representative population picture.

Glutamate uptake is another critical function of astrocytes, to reduce excitotoxicity from neuronal glutamate (92). As discussed reduced glutamate uptake is a feature of disease of the CNS, where it results in neuronal dysfunction and death (25, 63). In vitro glutamate uptake is typically measured by analysis of glutamate levels in cell media, which is now an easily accessible technique using commercially available kits.

Another characteristic of astrocytes is their trophic role in the CNS. Astrocytes secrete neurotrophic factors such as GDNF (104) and Clusterin (44), which promote neuronal survival. Release of such molecules can be detected by multiple methods, and has been demonstrated in hPSC-derived astrocytes (173). Alternatively, neurotrophic function can be evaluated in co-culture with neurons, or with 'astrocyte conditioned media' by examining their affect on neurons (44, 92, 173). These methods enable in vitro dissection of the supportive role of astrocytes and how this may be altered in the disease state.

One final aspect of astrocyte function is their response to injury, disease and signals from the immune system, which can all result in a reactive phenotype. Reactive astrocytes are key to disease in the CNS and have been shown to have both beneficial effects or to exacerbate the disease state. Therefore, we must be able to induce a reactive phenotype in vitro from hPSC derived astrocytes. This can influence all the aspects of astrocyte function we have discussed, but also has a key set of specific indicators. Induction can be achieved by many methods, including the addition of inflammatory cytokines (146) or introduction of toxins such as bacterial Lipopolysaccharides (66). First, we must be able to identify reactive astrocytes and, one feature is upregulation of GFAP as well as changes in astrocyte morphology. In addition reactive astrocytes demonstrate expression of specific markers such as intercellular adhesion molecule-1 (ICAM-1) (3, 5) or LCN2 (174), and the secretion of inflammatory cytokines such as IL-6 (146). Roybon and colleagues successfully induced and identified reactive astrocytes from hPSC derived cultures by exposing astrocytes to inflammatory cytokines, which induced markers of reactivity, and resulted in astrocytic secretion of IL-6 (146).

It is crucial that detailed identification and characterization criteria are applied to hPSC derived astrocytes. Together with the standardization of protocols this will to allow proper comparison across disease modeling studies, and provide a framework for the possible therapeutic application of these cells.

REFERENCES

1. Abbott NJ (2002) Astrocyte–endothelial interactions and blood–brain barrier permeability. J Anat 200:523–534.

2. Abramov AY, Canevari L, Duchen MR (2003) Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. J Neurosci 23:5088–5095.

3. Akiyama H, Kawamata T, Yamada T, Tooyama I, Ishii T, McGeer PL (1993) Expression of intercellular adhesion molecule (ICAM)-1 by a subset of astrocytes in Alzheimer disease and some other degenerative neurological disorders. Acta Neuropatholog 85:628–634.

4. Almad AA, Doreswamy A, Gross SK, Richard J-P, Huo Y, Haughey N, Maragakis NJ (2016) Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis. Glia 64:1154–1169.

5. Aloisi F, Borsellino G, Samoggia P, Testa U, Chelucci C, Russo G et al (1992) Astrocyte cultures from human embryonic brain: Characterization and modulation of surface molecules by inflammatory cytokines. J Neurosci Res 32:494–506.

6. Araque A, Parpura V, Sanzgiri RP, Haydon PG (n.d.) Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 22:208–215.

7. Ashley AK, Hinds AI, Hanneman WH, Tjalkens RB, Legare ME (2016) DJ-1 mutation decreases astroglial release of inflammatory mediators. NeuroToxicology 52:198–203.

8. Baarine M, Khan M, Singh A, Singh I (2015) Functional characterization of IPSC-derived brain cells as a model for x-linked adrenoleukodystrophy. PLoS One 10:e0143238.

9. Bales KR, Liu F, Wu S, Lin S, Koger D, DeLong C et al (2009) Human APOE isoform-dependent effects on brain -amyloid levels in PDAPP transgenic mice. J Neurosci 29:6771–6779.

10. Ballas N, Lioy DT, Grunseich C, Mandel G (2009) Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. Nat Neurosci 12:311–317.

11. Barmada SJ, Serio A, Arjun A, Bilican B, Daub A, Ando DM et al (2014) Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. Nat Chem Biol 10:677–685.

12. Barnabé-Heider F, Wasylnka JA, Fernandes KJL, Porsche C, Sendtner M, Kaplan DR, Miller FD (2005) Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1. Neuron 48:253–265.

13. Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60:430–440.

14. Barreto GE, Sun X, Xu L, Giffard RG (2011) Astrocyte proliferation following stroke in the mouse depends on distance from the infarct. PLoS One 6:e27881.

15. Bartheld von CS, Bahney J, Herculano-Houzel S (2016) The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. J Comp Neurol 524:3865–3895.

16. Bell KFS, Al-Mubarak B, Martel M-A, McKay S, Wheelan N, Hasel P et al (2015) Neuronal development is promoted by weakened intrinsic antioxidant defences due to epigenetic repression of Nrf2. Nat Commun 6:7066.

17. Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z et al (2012) Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. Nature 485:512–516.

18. Ben Haim L, Carrillo-de Sauvage M-A, Ceyzériat K, Escartin C (2015) Elusive roles for reactive astrocytes in neurodegenerative diseases. Front Cell Neurosci 9:278.

19. Bélanger M, Magistretti PJ (2009) The role of astroglia in neuroprotection. Dialog Clin Neurosci 11:281–295.

20. Bilican B, Serio A, Barmada SJ, Nishimura AL, Sullivan GJ, Carrasco M et al (2012) Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability. Proc Natl Acad Sci USA 109:5803–5808.

21. Bonni A, Sun Y, Nadal-Vicens M, Bhatt A, Frank DA, Rozovsky I et al (1997) Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway. Science 278:477–483.

22. Borghese L, Dolezalova D, Opitz T, Haupt S, Leinhaas A, Steinfarz B et al (2010) Inhibition of notch signaling in human embryonic stem cell-derived neural stem cells delays G1/S phase transition and accelerates neuronal differentiation in vitro and in vivo. Stem Cells 28:955–964.

23. Boyles JK, Pitas RE, Wilson E, Mahley RW, Taylor JM (1985) Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. J Clin Invest 76:1501–1513.

24. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211.

25. Bradford J, Shin JY, Roberts M, Wang CE, Li XJ, Li S (2009) Expression of mutant huntingtin in mouse brain astrocytes causes age-dependent neurological symptoms. Proc Natl Acad Sci USA 106: 22480–22485.

26. Brenner M, Johnson AB, Boespflug-Tanguy O, Rodriguez D, Goldman JE, Messing A (2001) Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. Nat Genet 27:117–120.

27. Briggs JA, Sun J, Shepherd J, Ovchinnikov DA, Chung TL, Nayler SP et al (2013) Integration-free induced pluripotent stem cells model genetic and neural developmental features of down syndrome etiology. Stem Cells 31:467–478.

28. Cahoy JD, Emery A, Kaushal A, Foo LC, Zamanian JL, Christopherson KS et al (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 28:264–278.

29. Caldwell MA, He X, Wilkie N, Pollack S, Marshall G, Wafford KA, Svendsen CN (2001) Growth factors regulate the survival and fate of cells derived from human neurospheres. Nat Biotechnol 19:475–479.

30. Carmeliet P, De Strooper B (2012) Alzheimer's disease: A breach in the blood–brain barrier. Nature 485:451–452.

31. Castelo-Branco G, Sousa KM, Bryja V, Pinto L, Wagner J, Arenas E (2006) Ventral midbrain glia express region-specific transcription factors and regulate dopaminergic neurogenesis through Wnt-5a secretion. Mol Cell Neurosci 31:251–262.

32. Cavanaugh SE (2014) Animal models of Alzheimer disease: historical pitfalls and a path forward. ALTEX 31:279–302.

33. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol 27:275–280.

34. Chandrasekaran A, Avci HX, Leist M, Kobolák J, Dinnyés A (2016) Astrocyte differentiation of human pluripotent stem cells: new tools for neurological disorder research. Front Cell Neurosci 10:41.

35. Chauhan A, Jeans AF (2015) Is Parkinson's disease truly a prion-like disorder? an appraisal of current evidence. Neurology Res Int 2015:1–8.

36. Chen C, Jiang P, Xue H, Peterson SE, Tran HT, McCann AE et al (2014). Role of astroglia in Down's syndrome revealed by patient-derived human-induced pluripotent stem cells. Nat Commun 5:180.

37. Chenn A (2009) A top-NOTCH way to make astrocytes. Dev Cell 16: 158–159.

38. Choi I, Choi D-J, Yang H, Woo JH, Chang M-Y, Kim JY et al (2016) PINK1 expression increases during brain development and stem cell differentiation, and affects the development of GFAP-positive astrocytes. Mol Brain 9:895.

39. Choi I, Kim J, Jeong H-K, Kim B, Jou I, Park SM et al (2013) PINK1 deficiency attenuates astrocyte proliferation through mitochondrial dysfunction, reduced AKT and increased p38 MAPK activation, and downregulation of EGFR. Glia 61:800–812.

40. Colombo JA, Reisin HD (2004) Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Res 1006:126–131.

41. Cookson MR (2010) The role of leucine-rich repeat kinase 2 (LRRK2) in Parkinson's disease. Nat Rev Neurosci 11:791–797.

42. Cookson MR (2015) LRRK2 pathways leading to neurodegeneration. Curr Neurol Neurosci Rep 15:44.

43. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW et al (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923.

44. Cordero-Llana O, Scott SA, Maslen SL, Anderson JM, Boyle J, Chowdhury R-R et al (2011) Clusterin secreted by astrocytes enhances neuronal differentiation from human neural precursor cells. Cell Death Differ 18:907–913.

45. Cramer PE, Cirrito JR, Wesson DW, Lee CYD, Karlo JC, Zinn AE et al (2012) ApoE-directed therapeutics rapidly clear b-amyloid and reverse deficits in AD mouse models. Science 335:1503–1506.

46. Crompton LA, Byrne ML, Taylor H, Kerrigan TL, Bru-Mercier G, Badger JL et al (2013) Stepwise, non-adherent differentiation of human pluripotent stem cells to generate basal forebrain cholinergic neurons via hedgehog signaling. Stem Cell Res 11:1206–1221.

47. Delépine C, Meziane H, Nectoux J, Opitz M, Smith AB, Ballatore C et al (2016) Altered microtubule dynamics and vesicular transport in mouse and human MeCP2-deficient astrocytes. Hum Mol Genet 25: 146–157.

48. Di Giorgio FP, Boulting GL, Bobrowicz S, Eggan KC (2008) Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. Cell Stem Cell 3:637–648.

49. Elisa Greggio MRC (2009) Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. ASN NEURO 1: AN20090007.

50. Elshourbagy NA, Boguski MS, Liao WS, Jefferson LS, Gordon JI, Taylor JM (1985) Expression of rat apolipoprotein A-IV and A-I genes: mRNA induction during development and in response to glucocorticoids and insulin. Proc Natl Acad Sci USA 82:8242–8246.

51. Emdad L, D'Souza SL, Kothari HP, Qadeer ZA, Germano IM (2012) Efficient differentiation of human embryonic and induced pluripotent stem cells into functional astrocytes. Stem Cells Dev 21:404–410.

52. Engel M, Do-Ha D, Muñoz SS, Ooi L (2016) Common pitfalls of stem cell differentiation: a guide to improving protocols for neurodegenerative disease models and research. Cell Mol Life Sci 73: 3693–3709.

53. Eroglu C, Barres BA (2010) Regulation of synaptic connectivity by glia. Nature 468:223–231.

54. Ertekin-Taner N (2007) Genetics of Alzheimer's Disease: A Centennial Review. Neurol Clin 25:611–667.

55. Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G et al (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. Hum Mol Genet 19:3053–3067.

56. Fan G, Martinowich K, Chin MH, He F, Fouse SD, Hutnick L et al (2005) DNA methylation controls the timing of astrogliogenesis through regulation of JAK-STAT signaling. Development 132:3345–3356.

57. Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W et al (2013) Toll-like receptor 4 is required for a-synuclein dependent activation of microglia and astroglia. Glia 61:349–360.

58. Ferreira M, Massano J (2017) An updated review of Parkinson's disease genetics and clinicopathological correlations. Acta Neurol Scand 135:273–284.

59. Funato H, Yoshimura M, Yamazaki T, Saido TC, Ito Y, Yokofujita J et al (1998) Astrocytes containing amyloid beta-protein (Abeta)-positive granules are associated with Abeta40-positive diffuse plaques in the aged human brain. Am J Pathol 152:983–992.

60. Gage FH (2000) Mammalian neural stem cells. Science 287:1433–1438.

61. Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W (2011) Astrocytes are important mediators of Ab-induced neurotoxicity and tau phosphorylation in primary culture. Cell Death Dis 2:e167.

62. Ge W, Martinowich K, Wu X, He F, Miyamoto A, Fan G et al (2002) Notch signaling promotes astrogliogenesis via direct CSL-mediated glial gene activation. J Neurosci Res 69:848–860.

63. Gil JM, Rego AC (2008) Mechanisms of neurodegeneration in Huntington's disease. Eur J Neurosci 27:2803–2820.

64. Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A et al (2006) Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. Neurobiol Dis 23:329–341.

65. Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ et al (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 86:7611–7615.

66. Guerra M, Tortorelli LS, Galland F, Da Ré C, Negri E, Engelke DS et al (2011) Lipopolysaccharide modulates astrocytic S100B secretion: a study in cerebrospinal fluid and astrocyte cultures from rats. J Neuroinflammation 8:128.

67. Gupta K, Patani R, Baxter P, Serio A, Story D, Tsujita T et al (2011) Human embryonic stem cell derived astrocytes mediate non-cell-autonomous neuroprotection through endogenous and drug-induced mechanisms. Cell Death Differ 19:779–787.

68. Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG (2007) Synaptic Islands Defined by the Territory of a Single Astrocyte. J Neurosci 27:6473–6477.

69. Hallmann A-L, Araúzo-Bravo MJ, Mavrommatis L, Ehrlich M, Röpke A, Brockhaus J et al (2017) Astrocyte pathology in a human neural stem cell model of frontotemporal dementia caused by mutant TAU protein. Sci Rep 7:42991.

70. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML et al (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41:1088–1093.

71. He F, Ge W, Martinowich K, Becker-Catania S, Coskun V, Zhu W et al (2005) A positive autoregulatory loop of Jak-STAT signaling controls the onset of astrogliogenesis. Nat Neurosci 8:616–625.

72. Henry AG, Aghamohammadzadeh S, Samaroo H, Chen Y, Mou K, Needle E, Hirst WD (2015) Pathogenic LRRK2 mutations, through increased kinase activity, produce enlarged lysosomes with reduced degradative capacity and increase ATP13A2 expression. Hum Mol Genet 24:6013–6028.

73. Herculano-Houzel S (2014) The glia/neuron ratio: How it varies uniformly across brain structures and species and what that means for brain physiology and evolution. Glia 62:1377–1391.

74. Herculano-Houzel S, Lent R (2005) Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. J Neurosci 25:2518–2521.

75. Hirabayashi Y, Gotoh Y (2010) Epigenetic control of neural precursor cell fate during development. Nat Rev Neurosci 11:377–388.

76. Hochstim C, Deneen B, Lukaszewicz A, Zhou Q, Anderson DJ (2008) Identification of positionally distinct astrocyte subtypes whose identities are specified by a homeodomain code. Cell 133:510–522.

77. Hong Y, Zhao T, Li X-J, Li S (2016) Mutant huntingtin impairs BDNF release from astrocytes by disrupting conversion of Rab3a-GTP into Rab3a-GDP. J Neurosci 36:8790–8801.

78. Hu J, Castets F, Guevara JL, Van Eldik LJ (1996) S100 beta stimulates inducible nitric oxide synthase activity and mRNA levels in rat cortical astrocytes. J Biol Chem 271:2543–2547.

79. Hu J-G, Lü H-Z, Wang Y-X, Bao M-S, Zhao B-M, Zhou J-S (2010) BMP signaling mediates astrocyte differentiation of oligodendrocyte progenitor cells. Tohoku J Exp Med 222:195–200.

80. Iadecola C, Nedergaard M (2007) Glial regulation of the cerebral microvasculature. Nat Neurosci 10:1369–1376.

81. Jenner P, Olanow CW (1996) Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 47:S161–S170.

82. Jones VC, Atkinson-Dell R, Verkhratsky A, Mohamet L (2017) Aberrant iPSC-derived human astrocytes in Alzheimer's disease. Cell Death Dis 8:e2696.

83. Juopperi TA, Kim W, Chiang C-H, Yu H, Margolis RL, Ross CA et al (2012) Astrocytes generated from patient induced pluripotent stem cells recapitulate features of Huntington's disease patient cells. Mol Brain 5:17.

84. Kamakura S, Oishi K, Yoshimatsu T, Nakafuku M, Masuyama N, Gotoh Y (2004) Hes binding to STAT3 mediates crosstalk between Notch and JAK–STAT signalling. Nat Cell Biol 6:547–554.

85. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O et al (12AD) Amyotrophic lateral sclerosis. Lancet 377:942–955.

86. Kim J, Basak JM, Holtzman DM (2009) The role of apolipoprotein e in Alzheimer's disease. Neuron 63:287–303.

87. Koehler RC, Roman RJ, Harder DR (2009) Astrocytes and the regulation of cerebral blood flow. Trends Neurosci 32:160–169.

88. Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J et al (2004) Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-b peptides. Nat Med 10:719–726.

89. Kondo T, Asai M, Tsukita K, Kutoku Y, Ohsawa Y, Sunada Y et al (2013) Modeling alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Ab and differential drug responsiveness. Cell Stem Cell 12:487–496.

90. Kondo T, Funayama M, Miyake M, Tsukita K, Era T, Osaka H et al (2016) Modeling Alexander disease with patient iPSCs reveals cellular and molecular pathology of astrocytes. Acta Neuropathol Commun 4:607.

91. Koyano F, Matsuda N (2015) Molecular mechanisms underlying PINK1 and Parkin catalyzed ubiquitylation of substrates on damaged mitochondria. Biochim Biophys Acta 1853:2791–2796.

92. Krencik R, Ullian EM (2013) A cellular star atlas: using astrocytes from human pluripotent stem cells for disease studies. Front Cell Neurosci 7:25.

93. Krencik R, Weick JP, Liu Y, Zhang Z-J, Zhang S-C (2011) Specification of transplantable astroglial subtypes from human pluripotent stem cells. Nat Biotechnol 29:528–534.

94. Kriks S, Shim J-W, Piao J, Ganat YM, Wakeman DR, Xie Z et al (2011) Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature 480:547–551.

95. Le Roux PD, Reh TA (1995) Astroglia demonstrate regional differences in their ability to maintain primary dendritic outgrowth from mouse cortical neurons in vitro. J Neurobiol 27:97–112.

96. Lee H, James WS, Cowley SA (2017) LRRK2 in peripheral and central nervous system innate immunity: its link to Parkinson's disease. Biochem Soc Trans 45:131–139.

97. Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S et al (2010) Direct transfer of -synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem 285:9262–9272.

98. Lev N, Barhum Y, Ben-Zur T, Melamed E, Steiner I, Offen D (2013) Knocking out DJ-1 attenuates astrocytes neuroprotection against 6-hydroxydopamine toxicity. J Mol Neurosci 50:542–550.

99. Li W, Cogswell CA, LoTurco JJ (1998) Neuronal differentiation of precursors in the neocortical ventricular zone is triggered by BMP. J Neurosci 18:8853–8862.

100. Li Y, Balasubramanian U, Cohen D, Zhang P-W, Mosmiller E, Sattler R et al (2015) A comprehensive library of familial human amyotrophic lateral sclerosis induced pluripotent stem cells. PLoS One 10:e0118266.

101. Liao MC, Muratore CR, Gierahn TM, Sullivan SE, Srikanth P, De Jager PL et al (2016) Single-cell detection of secreted A and sAPP from human IPSC-derived neurons and astrocytes. J Neurosci 36:1730–1746.

102. Lichtenberg M, Mansilla A, Zecchini VR, Fleming A, Rubinsztein DC (2011) The Parkinson's disease protein LRRK2 impairs proteasome substrate clearance without affecting proteasome catalytic activity. Cell Death Dis 2:e196.

103. Liebner S, Czupalla CJ, Wolburg H (2011) Current concepts of blood-brain barrier development. Int J Dev Biol 55:467–476.

104. Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260:1130–1132.

105. Liu H, Zhang S-C (2011) Specification of neuronal and glial subtypes from human pluripotent stem cells. Cell Mol Life Sci 68:3995–4008.

106. Lu CC, Brennan J, Robertson EJ (2001) From fertilization to gastrulation: axis formation in the mouse embryo. Curr Opin Genet Dev 11:384–392.

107. Lyst MJ, Bird A (2015) Rett syndrome: a complex disorder with simple roots. Nat Rev Gene 16:261–275.

108. Mabie PC, Mehler MF, Kessler JA (1999) Multiple roles of bone morphogenetic protein signaling in the regulation of cortical cell number and phenotype. J Neurosci 19:7077–7088.

109. Maclean GA, Menne TF, Guo G, Sanchez DJ, Park I-H, Daley GQ, Orkin SH (2012) Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. Proc Natl Acad Sci USA 109:17567–17572.

110. Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–630.

111. Mahley RW, Huang Y (2012) Apolipoprotein E sets the stage: response to injury triggers neuropathology. Neuron 76:871–885.

112. Majumder A, Dhara SK, Swetenburg R, Mithani M, Cao K, Medrzycki M et al (2013) Inhibition of DNA methyltransferases and histone deacetylases induces astrocytic differentiation of neural progenitors. Stem Cell Res 11:574–586.

113. Marchetto MCN, Muotri AR, Mu Y, Smith AM, Cezar GG, Gage FH (2008) Non-cell-autonomous effect of human SOD1G37R astrocytes on motor neurons derived from human embryonic stem cells. Cell Stem Cell 3:649–657.

114. Martin GR, Evans MJ (1975) Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. Proc Natl Acad Sci USA 72:1441–1445.

115. Matta S, Van Kolen K, da Cunha R, van den Bogaart G, Mandemakers W, Miskiewicz K et al (2012) LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. Neuron 75:1008–1021.

116. McGivern JV, Patitucci TN, Nord JA, Barabas M-EA, Stucky CL, Ebert AD (2013) Spinal muscular atrophy astrocytes exhibit abnormal calcium regulation and reduced growth factor production. Glia 61:1418–1428.

117. Miller RH, Raff MC (1984) Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct. J Neurosci 4:585.

118. Molofsky AV, Krencik R, Ullian EM, Tsai H-H, Deneen B, Richardson WD et al (2012) Astrocytes and disease: a neurodevelopmental perspective. Genes Dev 26:891–907.

119. Mori T, Koyama N, Arendash GW, Horikoshi-Sakuraba Y, Tan J, Town T (2010) Overexpression of human S100B exacerbates cerebral amyloidosis and gliosis in the Tg2576 mouse model of Alzheimer's disease. Glia 58:300–314.

120. Morrow T, Song M-R, Ghosh A (2001) Sequential specification of neurons and glia by developmentally regulated extracellular factors. Development 128:3585–3594.

121. Mullett SJ, Hinkle DA (2009) DJ-1 knock-down in astrocytes impairs astrocyte-mediated neuroprotection against rotenone. Neurobiol Dis 33:28–36.

122. Nagao M, Sugimori M, Nakafuku M (2007) Cross talk between notch and growth factor/cytokine signaling pathways in neural stem cells. Mol Cell Biol 27:3982–3994.

123. Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang H-Y (2003) Astrocytes accumulate A beta 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. Brain Res 971: 197–209.

124. Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M et al (1999) Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. Science 284:479–482.

125. Nakashima K, Yanagisawa M, Arakawa H, Taga T (1999) Astrocyte differentiation mediated by LIF in cooperation with BMP2. FEBS Lett 457:43–46.

126. Namihira M, Kohyama J, Semi K, Sanosaka T, Deneen B, Taga T, Nakashima K (2009) Committed neuronal precursors confer astrocytic potential on residual neural precursor cells. Dev Cell 16: 245–255.

127. Neumann M, Muller V, Görner K, Kretzschmar HA, Haass C, Kahle PJ (2004). Pathological properties of the Parkinson's disease-associated protein DJ-1 in alpha-synucleinopathies and tauopathies: relevance for multiple system atrophy and Pick's disease. Acta Neuropathologica, 107(6), 489–496.

128. Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553.

129. Palm T, Bolognin S, Meiser J, Nickels S, Träger C, Meilenbrock R-L et al (2015) Rapid and robust generation of long-term self-renewing human neural stem cells with the ability to generate mature astroglia. Sci Rep 5:16321.

130. Panatier A, Vallée J, Haber, M, Murai KK, Lacaille J-C, Robitaille R (2011) Astrocytes are endogenous regulators of basal transmission at central synapses. Cell 146:785–798.

131. Pannasch U, Freche D, Dallérac G, Ghézali G, Escartin C, Ezan P et al (2014) Connexin 30 sets synaptic strength by controlling astroglial synapse invasion. Nat Neurosci 17:549–558.

132. Patitucci TN, Ebert AD (2016) SMN deficiency does not induce oxidative stress in SMA iPSC-derived astrocytes or motor neurons. Hum Mol Genet 25: 514–523.

133. Pekny M, Pekna M (2016) Reactive gliosis in the pathogenesis of CNS diseases. Biochim Biophys Acta 1862:483–491.

134. Pellerin L, Bouzier-Sore A-K, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ (2007) Activity-dependent regulation of energy metabolism by astrocytes: an update. Glia 55:1251–1262.

135. Pihlaja R, Koistinaho J, Kauppinen R, Sandholm J, Tanila H, Koistinaho M (2011) Multiple cellular and molecular mechanisms Are involved in human Ab clearance by transplanted adult astrocytes. Glia 59:1643–1657.

136. Qian JA, Bull MS, Levitt P (1992) Target-derived astroglia regulate axonal outgrowth in a region-specific manner. Dev Biol 149:278–294.

137. Raff MC, Miller RH, Noble M (1983) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature 303:390–396.

138. Rannikko EH, Weber SS, Kahle PJ (2015) Exogenous a-synuclein induces toll-like receptor 4 dependent inflammatory responses in astrocytes. BMC Neurosci 16:57.

139. Reinhardt P, Glatza M, Hemmer K, Tsytsyura Y, Thiel CS, Höing S et al (2013) Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling. PLoS One 8:e59252.

140. Rossi D, Brambilla L, Valori CF, Roncoroni C, Crugnola A, Yokota T et al (2008) Focal degeneration of astrocytes in amyotrophic lateral sclerosis. Cell Death Differ 15:1691–1700.

141. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW et al (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16:675–686.

142. Rothstein JD, Martin LJ, Kuncl RW (1992) Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. N Engl J Med 326:1464–1468.

143. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW (1995) Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 38:73–84.

144. Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C (2008) Astroglial metabolic networks sustain hippocampal synaptic transmission. Science 322:1551–1555.

145. Rowland LP, Shneider NA (2001) Amyotrophic lateral sclerosis. N Engl J Med 344:1688–1700.

146. Roybon L, Lamas NJ, Garcia-Diaz A, Yang EJ, Sattler R, Jackson-Lewis V et al (2013) Human stem cell-derived spinal cord astrocytes with defined mature or reactive phenotypes. Cell Rep 4:1035–1048.

147. Sawaishi Y (2009) Review of Alexander disease: beyond the classical concept of leukodystrophy. Brain Dev 31:493–498.

148. Selkoe DJ (1986) Altered structural proteins in plaques and tangles: What do they tell us about the biology of Alzheimer's disease? Neurobiol Aging 7:425–432.

149. Serio A, Bilican B, Barmada SJ, Ando DM, Zhao C, Siller R et al (2013) Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. Proc Natl Acad Sci 110:4697–4702.

150. Shaked M, Weissmüller K, Svoboda H, Hortschansky P, Nishino N, Wölfl S, Tucker KL (2008) Histone deacetylases control neurogenesis in embryonic brain by inhibition of BMP2/4 signaling. PLoS One 3: e2668.

151. Shi Y, Kirwan P, Smith J, MacLean G, Orkin SH, Livesey FJ (2012) A human stem cell model of early alzheimer's disease pathology in down syndrome. Sci Transl Med 4:124ra29–124ra29.

152. Shin J-Y, Fang Z-H, Yu Z-X, Wang C-E, Li S-H, Li X-J (2005) Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. J Cell Biol 171:1001–1012.

153. Simpson JE, Ince PG, Haynes LJ, Theaker R, Gelsthorpe C, Baxter L et al (2010) Population variation in oxidative stress and astrocyte DNA damage in relation to Alzheimer-type pathology in the ageing brain. Neuropathol Appl Neurobiol 36:25–40.

154. Solano RM, Casarejos MJ, Menéndez-Cuervo J, Rodriguez-Navarro JA, García de Yébenes J, Mena MA (2008) Glial dysfunction in parkin null mice: effects of aging. J Neurosci 28:598–611.

155. Solano RM, Menéndez J, Casarejos MJ, Rodriguez-Navarro JA, Garcia de Yebenes J, Mena MA (2006) Midbrain neuronal cultures from parkin mutant mice are resistant to nitric oxide-induced toxicity. Neuropharmacology 51:327–340.

156. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388:839–840.

157. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci 90: 1977–1981.

158. Sun W, McConnell E, Pare JF, Xu Q, Chen M, Peng W et al (2013) Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. Science 339:197–200.

159. Sun Y, Nadal-Vicens M, Misono S, Lin MZ, Zubiaga A, Hua X et al (2001) Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. Cell 104:365–376.

160. Takizawa T, Nakashima K, Namihira M, Ochiai W, Uemura A, Yanagisawa M et al (2001) DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. Dev Cell 1:749–758.

161. Taylor JP, Brown RH, Cleveland DW (2016) Decoding ALS: from genes to mechanism. Nature 539:197–206.

162. Temple S (2001) The development of neural stem cells. Nature 414: 112–117.

163. Thal DR, Schultz C, Dehghani F, Yamaguchi H, Braak H, Braak E (2000) Amyloid beta-protein (Abeta)-containing astrocytes are located preferentially near N-terminal-truncated Abeta deposits in the human entorhinal cortex. Acta Neuropathol 100:608–617.

164. Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, Haustein MD et al (2014) Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nat Neurosci 17: 694–703.

165. Vallier L, Pedersen R (2007) Current Protocols in Stem Cell Biology, Vol. 448, pp. 191–1D.4.7. Hoboken, NJ, USA: Wiley.

166. Vallier L, Pedersen RA (2005) Human embryonic stem cells: an in vitro model to study mechanisms controlling pluripotency in early mammalian development. Stem Cells Rev 1:119–130.

167. Vallier L, Touboul T, Chng Z, Brimpari M, Hannan N, Millan E et al (2009) Early cell fate decisions of human embryonic stem cells and mouse epiblast stem cells are controlled by the same signalling pathways. PLoS One 4:e6082.

168. Weick JP, Held DL, Bonadurer GF, Doers ME, Liu Y, Maguire C et al (2013) Deficits in human trisomy 21 iPSCs and neurons. Proc Natl Acad Sci USA 110:9962–9967.

169. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA et al (2005) From the cover: Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc Natl Acad Sci USA 102:16842–16847.

170. Williams EC, Zhong X, Mohamed A, Li R, Liu Y, Dong Q et al (2014) Mutant astrocytes differentiated from Rett syndrome patients-specific iPSCs have adverse effects on wild-type neurons. Hum Mol Genet 23:2968–2980.

171. Wiseman FK, Al-Janabi T, Hardy J, Karmiloff-Smith A, Nizetic D, Tybulewicz VLJ et al (2015) A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome. Nat Rev Neurosci 16: 564–574.

172. Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F et al (2003) Adult mouse astrocytes degrade amyloid-[beta] in vitro and in situ. Nat Med 9:453–457.

173. Yamamizu K, Iwasaki M, Takakubo H, Sakamoto T, Ikuno T, Miyoshi M et al (2017) In vitro modeling of blood-brain barrier with human iPSC-derived endothelial cells, pericytes, neurons, and astrocytes via notch signaling. Stem Cell Rep 8:634–647.

174. Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32: 6391–6410.

175. Zhang P, Wang Q, Jiao F, Yan J, Chen L, He F et al (2016) Association of LRRK2 R1628P variant with Parkinson's disease in Ethnic Han-Chinese and subgroup population. Sci Rep 6:35171.

176. Zhang Y, Barres BA (2010) Astrocyte heterogeneity: an underappreciated topic in neurobiology. Curr Opin Neurobiol 20: 588–594.

177. Zhang Y, Sloan SA, Clarke LE, Caneda C, Plaza CA, Blumenthal PD et al (2016) Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. Neuron 89:37–53.

178. Zhou J, Su P, Li D, Tsang S, Duan E, Wang F (2010) High-efficiency induction of neural conversion in human ESCs and human induced pluripotent stem cells with a single chemical inhibitor of transforming growth factor beta superfamily receptors. Stem Cells 28:1741–1750.

179. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L et al (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 293:493–498.