Stem Cells / Review Article

Cells Tissues Organs

Cells Tissues Organs 2018;205:331–349
DOI: 10.1159/000493018

Accepted after revision: August 17, 2018
Published online: October 9, 2018

Advances and Current Challenges Associated with the Use of Human Induced Pluripotent Stem Cells in Modeling Neurodegenerative Disease

Bonnie J. Berry[a, b] Alec S.T. Smith[b, c] Jessica E. Young[a, b] David L. Mack[b–d]

[a]Department of Pathology, University of Washington, Seattle, WA, USA; [b]Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, USA; [c]Department of Bioengineering, University of Washington, Seattle, WA, USA; [d]Department of Rehabilitation Medicine, University of Washington, Seattle, WA, USA

Keywords

Induced pluripotent stem cells · Human stem cells · Neuron · Neurodegeneration · Alzheimer's disease · Maturation · Epigenetics

Abstract

One of the most profound advances in the last decade of biomedical research has been the development of human induced pluripotent stem cell (hiPSC) models for identification of disease mechanisms and drug discovery. Human iPSC technology has the capacity to revolutionize healthcare and the realization of personalized medicine, but differentiated tissues derived from stem cells come with major criticisms compared to native tissue, including variability in genetic backgrounds, a lack of functional maturity, and differences in epigenetic profiles. It is widely believed that increasing complexity will lead to improved clinical relevance, so methods are being developed that go from a single cell type to various levels of 2-D coculturing and 3-D organoids. As this inevitable trend continues, it will be essential to thoroughly understand the strengths and weaknesses of more complex models and to develop criteria for assessing biological relevance. We believe the payoff of robust, high-throughput, clinically meaningful human stem cell models could be the elimination of often inadequate animal models. To facilitate this transition, we will look at the challenges and strategies of complex model development through the lens of neurodegeneration to encapsulate where the disease-in-a-dish field currently is and where it needs to go to improve.

© 2018 S. Karger AG, Basel

Introduction

Neurodegenerative disease encompasses a broad range of conditions that primarily affect the human brain, resulting in progressive loss of function and eventual cell death. Diseases including amyotrophic sclerosis, Parkinson's, Huntington's, and Alzheimer's (AD) share similar challenges for robust modeling as they are all complicated, with pathologies associated with age, intricate genetics, and environmental factors. Model organisms from yeast to rodents have made important contributions to improving our understanding of key mechanisms that lead to neurodegenerative conditions, yet these models have failed to develop any drugs that prevent the progression of neurodegeneration across a wide range of diseas-

KARGER

E-Mail karger@karger.com
www.karger.com/cto

© 2018 S. Karger AG, Basel

Dr. David L. Mack
Department of Rehabilitation Medicine
University of Washington, 325 9th Avenue, Box 359612
Seattle, WA 98104 (USA)
E-Mail dmack21@uw.edu

es, including AD, Parkinson's disease, and Huntington's disease. Why? In many cases, animal models fail to recapitulate the complexity of human neurological states. For example, most animal models do not exhibit the extensive neuronal loss characteristic of advanced AD pathology [Grow et al., 2016; Eaton and Wishart, 2017]. Furthermore, the ability of rodents to model the motor impairment and perturbed decision-making characteristic of many neurodegenerative disorders is questionable due to differences in neuronal network development and complexity, reduced white matter content, and reduced neuronal density in targeted regions such as the substantia nigra [Grow et al., 2016]. The complete absence of brain regions containing gyrencephalic cortex, separate caudate and putamen or subthalamic nuclei further confounds the use of rodents to study human neurodegeneration [Eaton and Wishart, 2017].

Human stem cell technology, and induced pluripotent stem cells (iPSC) in particular, has made it possible to study *human* neurodegeneration from a developmental perspective while investigating the genetic contribution using gene-editing strategies. Next-generation sequencing techniques have synergized with high-throughput assays to enable the evaluation and comparison of multiple patient-specific cell lines, ushering in the possibility of precision medicine. Along with their enormous potential, neurological iPSC models come with several shortcomings that need to be acknowledged and overcome. Specifically, efforts are being made to improve retention of epigenetic profiles, reduce variability, increase maturity, and build multi-cell type organoids that mimic native tissue. Here we review each of these issues and how they pertain to the widespread adoption of hiPSC-based platforms in modeling human neurodevelopment and degeneration (Fig. 1). In addition, we discuss possible solutions to unsolved challenges and offer a new perspective on where efforts should be focused to further advance our understanding of, and ability to combat, neurodegenerative decline. We will attempt to answer the following question: what are the barriers between the current state of hiPSC technology and the full adoption of body-on-a-chip models of neuronal tissue?

Epigenetics

Human iPSC technology has unlocked new avenues of research in biomedical science. With the expression of just 4 factors, i.e., Oct4, Sox2, Klf4, and c-Myc, a terminally differentiated cell can be reverted into a pluripotent state [Takahashi and Yamanaka, 2006], making it possible to direct cells down almost any cell lineage. This groundbreaking discovery has been improved upon and alternative strategies have been developed [Mertens et al., 2016; Shahbazi et al., 2018], but the reprogramming process from somatic cell to iPSC remains inefficient [Zhou and Zeng, 2013; Schlaeger et al., 2015]. Part of this inefficiency is due to epigenetic barriers to this process that restrict the transition from terminally differentiated to pluripotent states [Ebrahimi, 2015]. Identification and utilization of novel methods which circumvent epigenetic barriers will be useful in improving reprogramming efforts and, ultimately, the robustness of human iPSC models. Overcoming these barriers and improving the precision of directed differentiations is an important problem to solve before stem cell transplant therapies can be adopted, as incompletely or inaccurately differentiated cells have the potential to create teratomas or other unpredictable effects [Hong et al., 2014].

Epigenetic modifications alter gene expression without changing the underlying DNA sequence. These epigenetic changes can be induced by many factors, including environment [Lopizzo et al., 2015; Holland et al., 2016], diet [Shimazu et al., 2013; Kong et al., 2017], exercise [Lavratti et al., 2017; Jessop and Toledo-Rodriguez, 2018], aging [Benayoun et al., 2015; Kubben and Misteli, 2017], disease [Christopher et al., 2017; Roubroeks et al., 2017], and cancer [Johnson et al., 2015; Toh et al., 2017]. Epigenetic regulation occurs through noncoding RNAs, direct chemical changes to DNA, or indirectly through DNA-associated proteins – all resulting in the suppression or enhancement of gene expression [Berger et al., 2009]. Multiple lines of evidence indicate the importance of epigenetic regulation in mediating reprogramming of somatic cells to iPSC. Specifically, large-scale shRNA screens have identified *Trim28* [Miles et al., 2017], *Setdb1* [Chen et al., 2013], and *Rif1* [Li et al., 2017] as repressors of epigenetic modification that can serve as barriers to reprogramming efforts through silencing of endogenous retroviruses involved in establishing pluripotency [Ivanov et al., 2007; Matsui et al., 2010; Karimi et al., 2011; Maksakova et al., 2011; Friedli et al., 2014; Ohnuki et al., 2014]. Knockdown of *Trim28* and *Setdb1* in wild-type and immortalized mouse embryonic fibroblasts has been shown to substantially increase reprogramming efficiency via a decrease in methylation of histone 3 lysine 9 (H3K9me3), a heterochromatic associated histone mark linked to repression of lineage genes [Hawkins et al., 2010; Becker et al., 2016; Miles et al., 2017]. Knockdown of *Rif1* similarly enhanced reprogramming efficiency. In

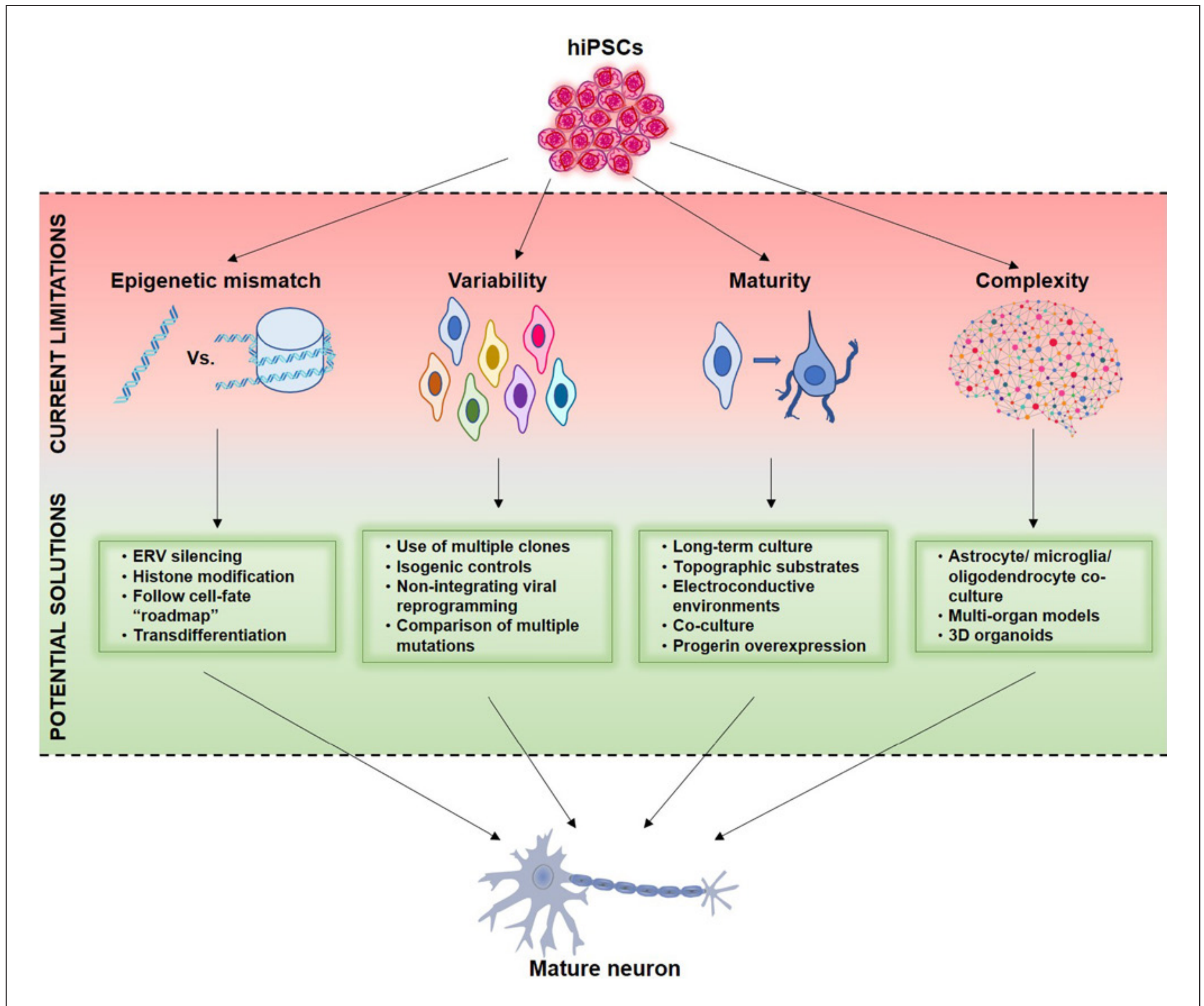

Fig. 1. Current limitations and potential solutions for producing hiPSC-derived neurons that match their in vivo counterparts as closely as possible.

addition to endogenous retroviruses repression regulators, histone-mediated chromatin structural states can also serve as a barrier to genetic reprogramming. MacroH2a isoforms have been shown to be incorporated into pluripotency genes to silence their activity and their removal may help facilitate reprogramming efforts [Gaspar-Maia et al., 2013].

Epigenetic silencing is especially important to consider when stem cells differentiate as the epigenetic switching of genes "on" or "off" is important in the regulation of cell fate [Ficz et al., 2011; Tao et al., 2018; Zhu et al., 2018]. This allows cells to develop distinct identities while maintaining a common genetic code. Reproducing gene-silencing patterns in vitro would be a powerful tool to recapitulate the in vivo niche to better study the normal function of individual cells and the development of disease states. An understanding of the epigenetic changes that neurons undergo during development is therefore beneficial when seeking to direct the differentiation of cells as close as possible to the desired cell type. To define the regulatory pathways that determine cell fate, Ziller et. al. [2015] tracked changes in DNA modification patterns,

an "epigenetic footprint," in human embryonic stem (ES) cells during neuronal differentiation. Analysis of global transcription in ES cells and 4 neural progenitor cell stages revealed the differential expression of 3,396 genes. DNA methylation data exhibited 2 overall patterns of epigenetic change in the transition from ES to a neural fate and beyond. The first pattern connected early neural fate decisions with the widespread loss of methylation and subsequent hypomethylation maintenance. The second pattern correlated later-stage cell fate decisions with a stage-specific DNA methylation loss and subsequent gain at the next stage. To identify the regulators of these patterns of epigenetic change, a computational model was developed to link epigenetic footprints to specific transcription factors. Analysis of the top-ranked transcription factors predicted to be activated during the cell state transition confirmed known regulators of neuronal development and differentiation, including PAX6, OTX2, FOXG1, and SOX proteins [Götz et al., 1998; Pevny et al., 1998; Martinez-Barbera et al., 2001; Hanashima et al., 2004] and neural induction pathways associated with inhibition of TGF-β and reduction in *SMAD4* activity [Chambers et al., 2009]. Such data may provide researchers with a "roadmap" for guiding the correct development of iPSC neurons for downstream applications.

Despite enormous efforts to engineer functional cells from iPSC that match as closely as possible the primary cells they are designed to phenocopy, iPSC-derived neurons do not always match their primary counterparts. While the genotypes may be the same and cells may display the correct surface markers and similar electrophysiological properties, differences can exist in the epigenetic profile between the primary cell and the reprogrammed mimic, resulting in significant variances in gene expression. It is not yet known what impact these differences will have when using hiPSC-derived neurons as predictors of human neural responses to chemical or pathological challenges. In a study of iPSC-derived midbrain mesodiencephalic dopaminergic neurons, major differences were found between primary cells and iPSC-derived neurons in levels of DNA methylation [Roessler et al., 2014]. These differences in methylation changed the expression levels of genes involved in function, neurodevelopment, regulation of synaptic transmission, and cell communication. Not surprisingly, gene expression patterns in iPSC-derived neurons strongly correlated with those found in embryonic primary neurons rather than postnatal-stage primary neurons. The iPSC-derived dopaminergic neurons displayed these differences in gene expression despite typical mature morphology, expression of archetypal markers (microtubule-associated protein 2 and tyrosine hydroxylase), and robust electrophysiological recordings. These observations highlight the importance of taking into consideration not only functional and morphological characteristics but also epigenetic characteristics.

Epigenetics in Disease

The fetal nature of iPSC-derived neurons presents a challenge when attempting to model advanced-age neurodegenerative disease [Studer et al., 2015], as results obtained in the context of late-onset degenerative disease may actually express biochemical markers more-closely associated with early-onset conditions. The epigenetic landscape is therefore important to consider when choosing a model system, particularly in studying human disease pathologies which occur at specific life stages. Transdifferentiation of aged cells may offer a suitable method to retain older epigenetic patterns when producing model neurons. Methods of direct conversion of fibroblasts into induced neurons have been developed in human models [Hu et al., 2015; Mertens et al., 2015]. Such methods of direct conversion have been shown to maintain multiple epigenetic age-associated signatures when converted from fibroblast to neuron [Huh et al., 2016]. However, transdifferentiation does have one significant drawback, since directly converted neurons are postmitotic and therefore cannot be expanded the way iPSCs can.

Other limitations to consider when recapitulating disease in hiPSC models are the side effects of cell expansion over time. For example, repeated passaging may pose a problem in modeling Rett syndrome, an X-linked neurodevelopmental disorder characterized by profound intellectual and motor disabilities caused by the loss of function in methyl-CpG-binding protein 2 [Ip et al., 2018]. Modeling this disorder in hiPSC is potentially problematic, as DNA methylation profiles, including those involved in X inactivation, may change during the repeated passaging of iPSCs [Bock et al., 2011; Nishino et al., 2011; Nazor et al., 2012]. Furthermore, analysis of low and high passage cultures of normal pluripotent populations has revealed the existence of hypermethylation events at CpG islands and silencing of genes, including *TSPYL5*. This tumor suppressor is downregulated in certain cancers, which could confound modeling of normal neurons [Weissbein et al., 2017]. The emergence of growth-promoting epigenetic alterations must therefore be carefully monitored, particularly when considering expanded, patient-derived cells for implant therapies.

In addition to improving hiPSC differentiation protocols, an understanding of how epigenetic misregulation causes disease may further our efforts to elucidate pathological mechanisms and treat complex neurological disorders using iPSC technology. In AD, misregulation occurs across all areas of epigenetic influence, including dysregulation in global methylation, altered expression of chromatin-associated proteins, and deregulation of noncoding RNA. Each aspect of misregulation is under intense scrutiny for potential therapeutic targets. Analysis of the epigenetic landscape in patients suffering from dementia has highlighted significant differences compared with age-matched controls, suggesting that alterations in DNA structure may play a central role in the progression of neurodegenerative conditions [Gräff et al., 2012]. In particular, global changes in hypo- and hypermethylation of CpG islands have been detected in dementia-associated brain regions. One study observed an approximately 20% decrease in DNA methylation markers 5-methylcytidine (5-mC) and 5-hydroxymethylcytidine (5-hmC) in hippocampal tissue from AD patients compared to nondemented, age-matched controls [Chouliaras et al., 2013]. Similar results were obtained from monozygotic twins discordant for AD [Chouliaras et al., 2013]. Identical DNA sequences producing discordant phenotypes with regard to dementia provide further evidence of the importance of noncoding genetic modification in neurodegeneration onset and progression. In another study of monozygotic twins discordant for AD, increased gene expression of sirtuin 1, an enzyme with a role in acetylation of DNA-associated proteins, as well as histone deacetylases (HDAC) HDAC2 and HDAC9, were observed in the AD-exhibiting twin [D'Addario et al., 2017]. In fact, expression of HDAC2, a known key player in functional plasticity in learning and memory, was previously shown to be upregulated in AD brains [Gräff et al., 2012] and current research is being focused on reducing HDAC protein expression as a target for the treatment of AD [Cuadrado-Tejedor et al., 2017; Yang et al., 2017; Volmar et al., 2018]. The role of noncoding RNA has been extensively reviewed elsewhere but includes influences on neuroinflammation, generation and clearance of amyloid-β (Aβ) and τ proteins, synaptic integrity and function, activity of mitochondria and endoplasmic reticulum, and the cell cycle [Maoz et al., 2017; Millan, 2017; Idda et al., 2018].

Not only is there evidence to suggest that epigenetic mechanisms contribute to AD pathology, but epigenetic mechanisms may also be responsible for exacerbating the damage caused by the disease and preventing endogenous processes that replace neuronal loss. Continual neurogenesis occurs in a healthy adult brain and helps maintain normal learning and memory through integration into the existing neuronal circuitry [Spalding et al., 2013; Ernst et al., 2014]. As discussed above, neurogenesis is mediated by epigenetic mechanisms that tightly control gene expression during cell fate decisions. Though controversial [Toda et al., 2018], these processes may be impaired in the AD brain [Boekhoorn et al., 2006; Li et al., 2008], and the loss of neurons without replacement would certainly accelerate disease progression. Conflicting results regarding the occurrence of neurogenesis in AD may be due to a lack of information regarding the occurrence of neurogenesis at specific disease stages or the variant nature of AD progression.

As our understanding of the epigenetic landscape and its role in neurodegenerative disease improves, our capacity to manipulate such changes to combat disease likewise increases. For example, high-throughput screening of neural progenitors has been used to identify compounds capable of reversing epigenetic silencing of the *Fmr1* gene in fragile X syndrome. Mental retardation in fragile X is largely caused by epigenetic silencing of Fmr1 and these results offer hope that targeted reversal of these pathogenic epigenetic modifications may be possible in the near future [Kaufmann et al., 2015].

Variability

Research involving animal models relies heavily on inbred lines to ensure consistency both internally within a given study and externally between studies conducted at different sites. The use of animal lines with identical genetic backgrounds provides a degree of certainty that the differences observed have arisen from experimental manipulation rather than differential responses due to genetic variation. Clonal human iPSC lines also offer consistency of genotype. However, genetic variability across human populations means that substantial differences between lines exist, and the means to account for this heterogeneity when predicting patient responses to therapeutic interventions have yet to be established. Personalized medicine – the means to conduct studies on a patient's own cells to determine the best possible therapeutic options for that individual – holds great potential to improve standards of care and reduce costs in the long run, but our current inability to fully exploit iPSCs for such applications further underscores the difficulty in using these cells to test basic biological hypoth-

Cells Tissues Organs 2018;205:331–349
DOI: 10.1159/000493018

eses and address the pathophysiological variability across human populations.

In the past, issues have been raised regarding the persistence of cell-of-origin memory after a somatic cell type has undergone iPSC reprogramming [Bar-Nur et al., 2011]. However, long-term culture and serial reprogramming have proven efficacious in erasing such memory [Kim et al., 2010; Polo et al., 2010], as well as reducing differences between iPSC and ES cell lines [Chin et al., 2010] and eliminating genetic mosaicism [Hussein et al., 2011]. Worries about the incorporation of reprogramming factor genes into the iPSC genome, thereby potentially leading to alterations in the genotype, have also been raised. Fortunately, the generation of replication-defective persistent Sendai virus has enabled effective cellular reprogramming followed by erasure of the vector's genomic RNA [Nishimura et al., 2011], reducing the risk of variability or spurious gene activation/inactivation in companion iPSC lines. Using such vectors, it has been shown that donor cell type contributes minimally to iPSC variability in terms of gene expression profiles and the epigenetic landscape [Kyttälä et al., 2016]. However, this same study, along with others [Rouhani et al., 2014], has highlighted that donor-related variability can influence expression of lineage-priming genes in iPSCs. This suggests that such cells exhibit a variable capacity to differentiate down specific lineages and that this is dictated, at least in part, by the donor genotype. Therefore, the ability to produce a fully differentiated, functional somatic cell from iPSC is determined not only by the efficiency of the differentiation method employed but also by the parent donor cell used to generate the iPSC line. This makes heterogeneity downstream of a given differentiation method likely when comparing multiple iPSC sources, which suggests that direct comparisons of a given cell type's response to challenge across different genetic backgrounds may be problematic and strongly argues for using isogenic iPSC lines whenever possible.

Within one congenital disease, further variability exists in the number of causal genes and the range of pathogenic mutations in each gene known to produce symptoms. Even for relatively straightforward, monogenic disorders, different mutations cause variable pathological phenotypes depending on whether the location of the mutation is in the structural or regulatory portion of the gene. For example, Huntington's disease is caused by the presence of a mutation for a long series of glutamine-encoding CAG repeats (>36 repeats) in the Huntingtin (*HTT*) gene, and longer repeat sequences correlate with a younger age of disease onset [Andrew et al., 1993]. The length of CAG repeats in the *HTT* sequence of patient-derived iPSC has likewise been shown to produce line-specific phenotypes when these cells are differentiated into neurons [Berndt et al., 2011]. Such line-specific differences include changes in energy metabolism, cell-cell adhesion, stressor vulnerability, and cell survival, with longer CAG repeats producing poorer readouts for these metrics.

AD can be broadly broken down in to early onset (prior to age 65 years) and late onset (after age 65 years). Both early-onset AD and late-onset AD have a heritable component, with the majority of early-onset AD showing a strong family history [Bekris et al., 2010]. Autosomal dominant AD is almost exclusively early-onset AD and accounts for approximate 1% of all AD cases [Bekris et al., 2010]. This form of the disorder is due to more than 200 changes that have been reported in the genes encoding amyloid precursor protein (*APP*), presenilin (PSEN) 1, and PSEN 2 [Ryan and Rossor, 2010]. Although the pathogenic nature of these changes has not been confirmed in every instance, this heterogeneity underscores the significant variability in patients suffering from this debilitating condition. Even assuming that only a subset of these mutations are pathological, mutations in these genes account for a small percentage of AD in general, and the degree to which the study of these mutations can serve as a paradigm for late-onset sporadic AD, which has complex genetic and environmental components, is still uncertain. In terms of late-onset sporadic AD, the *APOE* gene has been most consistently associated with AD risk. In humans, there are 3 isoforms of APOE with polymorphisms encoding either cysteine or arginine at amino acid positions 112 and 158. The frequency of the APOE e4 allele, coding for arginine, is highly increased in patients with AD and the presence of the arginine amino acid affects the structure and lipid-binding abilities of the APOE protein [Bekris et al., 2010]. Recent work using isogenic cellular models of APOE derived from hiPSCs has yielded exciting data indicating that APOE e4 increases Aβ production and τ phosphorylation (the 2 main pathological hallmarks of AD) and also causes gene expression changes in brain inflammatory cells, suggesting that upregulation of an innate immune response in the brain can contribute to neurodegeneration [Lin et al., 2018; Wang et al., 2018a]. Importantly, these effects were seen exclusively in human cells, highlighting the differences between hiPSC and other commonly used nonhuman model systems. Despite this, comparing diseased cells with those from healthy controls can raise issues with baseline genetic variance between healthy and patient genotypes. The in-

creasing availability of CRISPR/Cas9 and other gene-editing methods has made the generation of isogenic cell lines, where different variant lines are derived from the same genetic background, a critical component for investigating the potential pathogenic effects of a given variant. By correcting the mutation in a given line, or inducing a pathological mutation in an otherwise healthy line, researchers can study the effect of that mutation against controls without worrying about donor variance. However, off target effects of the editing process cannot be ruled out. In particular, activation of a DNA damage response via p53 activation has recently been reported to account for Cas9 toxicity in pluripotent stem cells [Ihry et al., 2018]. At this point, it is also unknown if off target effects have a significant impact on the eventual phenotype of differentiated cells. Efforts to improve the targeting efficiency of current gene-editing methods are an active area of current research [Miller et al., 2007; Sanjana et al., 2014; Tsai et al., 2014], but the genome-wide specificities of these techniques have yet to be fully defined. The importance of understanding the unexpected impact of gene editing on iPSC-derived somatic cell function within the scientific community is clear from the recent establishment of several initiatives, including the US NIH Somatic Cell Genome Editing (SCGE) program [National Institutes of Health, 2018], which is seeking to fund projects aimed at defining the risk of adverse biological consequences of current editing methods, including CRISPR/Cas9. Likewise, the European Academies Science Advisory Council published a report last year in which they emphasized the need for "intensive basic and clinical research" to better understand "the risks such as inaccurate editing and the potential benefit" of proposed genome modifications on human cells, for both clinical and nonclinical applications [European Academies' Science Advisory Council, 2017]. Given the potential impact of such technologies for combatting genetic disorders, we can expect such research to proceed rapidly in the coming years, leading to a greater understanding of how such methods could impact the reliability of iPSC-based research.

Clonal variability represents one final source of potential variance in iPSC-based research. Investigations into mutational load in iPSC lines have demonstrated that there is a significant risk for iPSCs to exhibit new mutations as a result of reprogramming, but that new mutations vary in different iPSC clones [Gore et al., 2011]. The authors of this work admit the possibility that reprogramming itself is "mutagenic" but provide data arguing that selection during reprogramming, colony-picking, and subsequent culture may be contributing factors. As such, it is possible that if reprogramming efficiency is improved to the point where no colony picking or clonal expansion is required then the resulting iPSC may have a significantly reduced incidence of new mutations. Several studies have reported significant copy number alterations, including duplications and deletions, between iPSC and their donor cells [Laurent et al., 2011; Martins-Taylor et al., 2011; Kilpinen et al., 2017]. Most of these alterations appear to be iPSC clone specific, leading to genetic mosaicism in cultures established from nonclonal colonies [Kilpinen et al., 2017]. In fact, one study of 711 iPSC lines derived from 301 healthy individuals found that just 15% of the observed copy-number alterations were identified in all replicates [Kilpinen et al., 2017], highlighting the significant potential for genotype variability in different iPSC clones. Despite these findings, recent work has highlighted that the widespread use of more than one clone per individual can be detrimental to the robustness of the findings and that spurious differential expression is exacerbated by the inclusion of multiple iPSC clones for a given line [Germain and Testa, 2017]. As such, the authors recommend increasing the number of donor lines, rather than the number of clones, to improve assay sensitivity.

Additionally, the ability to detect genomic instability in stem cell populations is crucial to moving forward with in-patient therapeutic applications. In 2015, safety concerns prevented the progression of the first-in-human clinical trial of patient-derived stem cells [Chakradhar, 2016]. Patient fibroblasts were reprogrammed to retinal neurons and transplanted for the treatment of age-related macular degeneration. No genetic abnormalities were detected in the first patient's cells. She was not given any immunosuppressants, and her worsening vision stabilized. However, the second patient's screen for genetic abnormalities revealed mutations in 3 genes. One of the 3 genes is known as an oncogene in the Catalogue of Somatic Mutations in Cancer. Despite the fact that that these mutations were not shown to lead directly to adverse effects, the planned transplantation of an hiPSC-derived retinal pigment epithelium was cancelled, highlighting the need for a greater understanding of the risks posed by novel iPSC mutations.

Although considerable work has been done to characterize the level of genetic variability that exists in and between iPSC populations, no methods have yet been proposed to assess the experimental significance that such variability could impose on the study of neural development and pathology or on potential clinical outcomes of hiPSC-based therapeutics. Increasing the number of lines

Cells Tissues Organs 2018;205:331–349
DOI: 10.1159/000493018

used within a given study and (possibly) the number of iPSC clones analyzed per line may help account for variability in differentiation efficiency. However, how many lines would be necessary to account for any observed variance has not been widely agreed upon and would likely vary depending on the specific requirements of the study in question. High-throughput screening modalities could offer the means to compare large iPSC libraries for a given effect or drug response. However, in certain cases with substantial patient variability, whether the number of lines necessary to enable extrapolation of conclusions that pertain to a meaningful subset of the human population would be practical for the majority of research laboratories is an open question.

Maturity

Human iPSC should, in theory, mimic exactly the phenotype of ES cells [Robinton and Daley, 2012]. As such, differentiation methods developed to produce somatic cells from these sources should attempt to mirror the developmental signaling cascade known to regulate lineage specification during embryo patterning and differentiation. As our understanding of these pathways improves, our capacity to control iPSC differentiation likewise increases. For example, recent work studying the differentiation of skeletal muscle has led to the development of methods to produce functional muscle fibers from both healthy and dystrophic patient-derived iPSCs [Chal et al., 2015, 2016; Xi et al., 2017; Hicks et al., 2018]. Although neuronal and cardiac differentiation methods have been available for some time [Zhu et al., 2011; Selvaraj et al., 2012], our ability to produce skeletal muscle has lagged behind and it is only with improved understanding of the necessary developmental cues provided in utero that we have been able to further our capacity to produce such lineages from iPSC in vitro. Transcriptomic studies of primary and iPSC-derived cortical neurons have confirmed that iPSC-derived neurons are highly similar to primary cortical neurons at the level of single cells. However, some data suggests that current markers may not be able to disambiguate cortical layer identity in these cultures [Handel et al., 2016]. Therefore, our ability to produce mixed populations of cortical neurons from iPSCs is well established but our capacity to identify specific cortical subtypes requires further refinement.

Despite our ever-increasing ability to produce specific somatic cell types and subtypes, the vast majority of iPSC-directed differentiations produce populations that most closely resemble fetal or neonatal cells. In order to effectively model adult physiological responses to chemicals or adult onset diseases, there remains a need to devise practical methods to promote maturation of cells differentiated from iPSCs to the point where clinically-predictive data can be reliably obtained. During embryogenesis, the development of synaptically active, postmitotic neurons from early-stage neural precursors depends on a tightly regulated transcriptional cascade. The resulting morphological changes then enable the connectivity of the synaptic network necessary to facilitate sustained central nervous system function [Dodla et al., 2010; Kaur et al., 2014]. Similarly, the functional properties of emerging iPSC-based neuronal networks in vitro are highly changeable and subject to differentiation efficiency, cellular subtype specification, and maturation of the neurons, as guided by the input cues provided by researchers. Cultures derived from primary tissues have been shown to readily form functional networks in vitro [Biffi et al., 2013; Penn et al., 2016], and stem cell-based models have likewise been proven capable of exhibiting similar connectivity in culture [Ban et al., 2006]. However, the extent to which these networks are capable of modeling connectivity in the aged brain has yet to be fully characterized and it seems likely that in situ maturation of iPSC-derived neuronal populations would likely improve our ability to study age-associated neurodegeneration using such systems. The necessity to further mature iPSC-derived somatic cells has been widely discussed in the cardiac literature, to the point where several groups have detailed criteria that should be considered when gauging the maturation state of iPSC-derived cardiac populations to ensure their suitability for use in predictive preclinical assays [Lundy et al., 2013; Sheehy et al., 2014; Yang et al., 2014b; Kolanowski et al., 2017]. However, a comprehensive list of maturation criteria with which to assess neuronal development in vitro has been less forthcoming. The importance of developing mature neurons is clear, given the number of degenerative neurological conditions that are associated with advanced age [Deecke and Dal-Bianco, 1991], but what criteria should be used to assess iPSC-derived neuronal maturation? In Table 1 we provide a list of potential metrics and justification for gauging in vitro neuron maturity. Such a list could serve as the basis for more standardized assessment of neuronal maturity prior to use in downstream applications.

The defining properties of an electrically-active cell type are its ability to fire an action potential (AP) and respond correctly to neurotransmitter release. Functional output is thus the gold standard for assessing the health

Table 1. Approaches for evaluating the functional maturity of neurons maintained in vitro and the significance of each metric

Phenotypic feature	Parameter	Method of study	Metrics	Significance	References
Electrophysiology	Resting membrane potential and intrinsic membrane properties	Patch clamp	Human neurons in vivo exhibit a resting membrane potential of –70 mV; mature neurons should mimic this as closely as possible while also exhibiting reduced AP threshold and input resistance	Measure of the ionic gradient necessary for generation of an AP; becomes more negative during development. AP threshold occurs at -40 mV in vivo; recapitulation of these values ensures that functional responses to treatment are more likely to be representative of the in vivo condition	Ambasudhan et al., 2011; Takazawa et al., 2012; Wainger et al., 2014; Lam et al., 2017
	Repetitive firing capacity	Patch clamp	Increased frequency of depolarizing spikes in response to current injection; >10 Hz is typical in mature neurons	Cells capable of repetitive firing have a sufficient density of ion channels and ATP pumps to quickly and efficiently repolarize the membrane after an AP is fired; necessary for functional network performance	Takazawa et al., 2012; Sun et al., 2018
	Inward current/current density	Patch clamp	>500 pA inward currents typically observed in mature neurons	Increased sodium and potassium channel expression in more mature neurons leads to larger inward and outward currents and a greater current density, which in turn implies a greater excitability of the membrane	Gu and Haddad, 2003; Schaarschmidt et al., 2009
	Burst firing	MEA	Spike frequency in the range of 6–10 Hz and synchronized burst firing events at 3–6 per min	Synchronized burst firing is indicative of the formation of robust synaptic networks in cultured neurons; routine measurement of synchronous activity across multiple electrodes suggests a well-established interconnectivity	Biffi et al., 2013; Odawara et al., 2016
Morphology	Axon specification	Phase imaging/ICC	τ-1, PMGS, and TrkA localization, increased outgrowth rate (3× other neuritic extensions)	Neuritic extension and specification into axons constitutes a critical step in normal neuron morphology development	Jiang and Rao, 2005; Wiggin et al., 2005; Yamamoto et al., 2012
	Dendritic arborization	Phase imaging/ICC	Increased dendrite length, number, spine head volume, and density as cells mature	Increased branching in neuronal dendrites provides for more intricate synaptic networks and a greater degree of functional connectivity	Harris et al., 1992; Konur et al., 2003; Bourne and Harris, 2008
	Density of synaptic puncta	ICC	Increased synaptic density as cells mature	Synaptic density dictates neuronal network complexity and interconnectivity; a greater density of synaptic puncta therefore indicates an increased capacity for network activity in such cultures	Cullen et al., 2010
Transcriptome/ proteome	Gene expression	Protein/mRNA detection	Absence of early (e.g., Nestin, Sox1, Sox2), and presence of mature neuronal markers (layer/cell type specific; e.g., CTIP2, tyrosine hydroxylase); combined electrophysiology and single cell transcriptome analysis has identified the mitochondrial electron transport genes NDUFC2 and UQCRB, as well as UCHL1, BEX2, PDGFA, FAIM2, CNTN1, SEC62, ATP1B1, and CALM2, as indicative of a mature neuronal phenotype	Movement towards transcriptome profiles that more closely mimic those of mature neurons provides greater confidence that neuronal responses to chemical or pathological challenge in vitro will offer more representative results in terms of recapitulating human tissue responses	Chen et al., 2016; He and Yu, 2018
Metabolism	Mitochondrial movement	Mitochondrial movement-tracking assays	In mature neurons, roughly 20–30% of axonal mitochondria are motile, while 15% either briefly pause or dock at synapses	While mitochondria in young neurons are functionally similar to mature neurons, they exhibit enhanced motility, perhaps to permit faster energy dispersal for cellular demands, such as synaptogenesis; as cells mature, mitochondria in the processes then elongate and reduce their motility for long-term support of synaptic structures	Chang and Reynolds, 2006; Lin and Sheng, 2015; Lewis et al., 2016

ICC, immunocytochemistry; AP, action potential; MEA, microelectrode array.

and maturity of neurons and can be measured via patch clamp electrophysiology [Franz et al., 2017], microelectrode array [Obien et al., 2014], or application of voltage-sensitive dyes [Mennerick et al., 2010]. Regardless of the AP properties of individual neurons, the predictive value of iPSC-based central nervous system models is predicated on the ability of the derived cells to recapitulate accurately the functional behaviors of primary neuronal networks through synaptic connectivity. Neurons derived from human iPSCs have been shown to fire AP in response to depolarizing current injections after just 3 weeks of differentiation [Prè et al., 2014]. However, the AP properties at this time point are relatively poor, but in the following weeks they undergo significant improvements characterized by increases in Na^+ and K^+ current amplitudes, AP amplitude, full width at half maximum of the AP spike, and AP threshold [Prè et al., 2014]. Resting membrane potential in these cells likewise decreased with time, illustrating an improved capacity for cells to regulate ion gradients across their membranes with increased time in culture.

Time has likewise proven to be a key regulator of network connectivity in iPSC neuronal models. Analysis of 1-year-old hiPSC-derived cerebral cortical neurons on microelectrode arrays has illustrated that synchronized burst firing (simultaneous AP bursts) behavior increased steadily from 16 to 30 weeks in culture and that the response to glutamatergic synaptic agonists and inhibitors was similarly time dependent [Odawara et al., 2016]. Also, spike frequency appeared to plateau after 23 weeks in culture, whereas synchronized burst activity steadily increased up to 30+ weeks. Given the differences in firing behavior and drug responses at different time points, these data highlight the need to establish suitable endpoints for pharmacological studies, as compound efficacy will likely vary with culture age. It also seems probable that rate of functional development in iPSC-derived neuronal populations will depend on donor genotype (as discussed above) so the need to establish functional criteria with which to normalize performance and establish experimental endpoints is high. Quantifying AP parameters, resting membrane potentials, spontaneous AP firing rates, burst fire activity, and network burst activity and establishing endpoints when these metrics reach a plateau seems logical. However, culture periods of 30+ weeks are likely impractical for a wide number of downstream assays.

Long-term culture coupled with functional analysis may also prove efficacious in the study of neurodegenerative disease. A recent study utilizing commercially sourced iPSC-derived cortical neurons investigated functional responses to acute supraphysiological doses of Aβ in comparison with low dose physiological concentrations of the protein [Berry et al., 2018]. The authors aimed to determine whether chronic exposure to Aβ at levels comparable to those observed in patients produced a different phenotype compared to the short-term high dose often used in vitro when studying AD. The results suggested that extended, low doses of Aβ produced mild functional impairment of the iPSC-derived neurons without the excessive levels of cell death seen with acute, high doses. Therefore, it is possible that a more measured and physiologic approach to the study of Aβ on neuronal function may enable assessment of early cognitive impairment instead of the catastrophic impact of supraphysiological doses applied in the short term. However, the degree to which the functional impairment observed mirrors the functional capacity of neurons in patients with mild cognitive impairment remains to be seen. In another example, researchers investigated the firing capabilities of motor neurons harboring *TARDBP* or *C9ORF72* amyotrophic lateral sclerosis-causing mutations and showed time-dependent changes in firing capacity [Devlin et al., 2015]. Mutant cells were found to exhibit a progressive decreasing ability to fire chains of AP in response to depolarizing current injections between 4 and 10 weeks in vitro, suggesting that extended culture periods may be necessary to model functional decline in neurodegenerative disease.

In addition to functional performance, morphological characteristics should be considered when assessing neuronal development in vitro. Long-term culture has again shown significant increases in the soma size and primary dendrite widths of developing neurons [Odawara et al., 2014]. The density of synaptic junctions formed on cultured neurons also offers a strong indicator of the structural development of a given neuronal population as well as the degree of connectivity. Complex dendritic arborization and axonal distinction [Gouder et al., 2015; Kang et al., 2017] coupled with stable expression of pan-neuronal markers such as NeuN, MAP-2, and Tuj1 [Hu et al., 2010; Gunhanlar et al., 2018] should likewise be considered.

Despite the positive contribution of long-term culture, it may be impractical for many applications. In addition, it is not yet known whether neurons cultured in vitro for extended periods will *ever* reach the point of accurately recapitulating the environment of an aged and/or demented brain. It is clear that other methods to drive neuronal maturation in vitro are warranted. Topographic

substrates mirroring the structural cues provided by the extracellular matrix have been shown to influence neuronal differentiation from iPSC, with specific topographies altering yes-associated protein expression and in turn affecting the neural differentiation capacity of cultured cells [Song et al., 2016]. Topographic surfaces have also been shown to enhance direct conversion of fibroblasts to neurons through modulation of histone modifications related to cytoskeletal organization [Yoo et al., 2015]. Such substrates enable control of neuritic outgrowth and polarity, which may also offer benefits when seeking to develop controlled neuronal networks [Ferrari et al., 2011]. Studies into other electrically active cell types, like cardiomyocytes, have demonstrated that topographic patterning can enhance physiological structure and function [Yang et al., 2014a; Carson et al., 2016] but whether or not such surfaces enhance network connectivity and functional performance in iPSC-based neuronal cultures remains to be seen. Highly conductive substrates have been shown to improve network activity in cultured neuronal populations [Lovat et al., 2005; Tang et al., 2013]. Although the exact mechanism behind this phenomenon has yet to be characterized, the collected data demonstrate the ability for such conductive surfaces to enhance functional parameters of neurons in vitro.

To model age-related disorders, researchers have shown that forced overexpression of progerin in iPSC-derived neurons can reestablish age-related markers lost during reprogramming and that the resulting cells exhibit degenerative changes in dendrite branching, protein folding, and oxidative stress [Miller et al., 2013]. Furthermore, such genetic manipulation in iPSCs derived from Parkinson's disease patients produced neurons with condensed nuclei that express cleaved caspase-3. Progerin overexpression in these neurons exacerbated the loss of dendrite length when compared with non-Parkinson's control cells. Alternatively, manipulation of telomerase expression in iPSC-derived neurons, through exposure to the telomerase inhibitor BIBR1532, reduced dendritic development and led to a reduction in the expression of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of dopamine, a phenotypic feature associated with Parkinson's disease [Vera et al., 2016]. Such methods indicate that manipulation of genetic regulators of aging are a viable means of achieving an aged neuronal phenotype. However, direct reprogramming (transdifferentiation; discussed above) may offer an alternative method to achieve mature neurons without reliance on complex or protracted maturation protocols. Using transdifferentiated neurons, researchers have identified the nuclear pore-associated transport protein RanBP17 as a significant factor in cell aging, both in neurons and other cell types, and demonstrated that direct conversion of aged fibroblasts to neurons enabled retention of age-related nucleocytoplasmic defects associated with this protein [Mertens et al., 2015]. Direct conversion of fibroblasts derived from familial AD patients produced neurons with elevated levels of Aβ and phosphorylated τ compared with controls [Hu et al., 2015], indicating that induced neurons can also recapitulate AD phenotypes in cultured neurons.

Complexity

As valuable as hiPSC-derived neurons are for studying specific cellular mechanisms, purified cultures of individual neuronal subtypes do not represent the in vivo niche and will never fully recapitulate the complexity of the human central nervous system. As such, their capacity to accurately model neuronal circuitry and degenerative behavior in disease states is limited. As our ability to generate mature subtype-specific neurons improves, our capacity to create more and more complex in vitro systems likewise increases. Such technological advances could enable more predictive study of human neurological and pathological phenomena, thereby enhancing the development of novel therapeutics and eventually removing ethically questionable and unreliable animal models all together. Here we describe recent efforts to create more complex multicell-type models incorporating hiPSC-derived neuronal elements and describe their uses in studying nervous system development and disease.

Use of multielectrode arrays has shown that the electrophysiological properties of hiPSC-derived neurons improve over time and that the level of spontaneous activity is increased through coculture with rodent astrocytes [Odawara et al., 2014]. Specifically, spontaneous firing rates in coculture on day 35 were twice the level reported in neuron-only controls, and mixed populations could be maintained for more than 120 days. More recently, coculture of hiPSC-derived cortical neurons with human astrocytes showed more frequent and synchronized spike trains as well as more dynamic changes in overall spike patterns compared with neuron-only controls [Kuijlaars et al., 2016; Kayama et al., 2018]. The occurrence of temporally coordinated spiking patterns may represent physiological signs of organized circuits within the cultured hiPSC-derived neuronal population, indicating the value of astrocytes on neuronal organization

Cells Tissues Organs 2018;205:331–349
DOI: 10.1159/000493018

and function in vitro. In addition to improving baseline function, astrocytes have been shown to effectively rescue defects in neurogenesis of dopaminergic neurons exhibiting mitochondrial respiratory chain disruption [Du et al., 2018]. In this system, coculture of astrocytes with defective neurons restored mitochondrial functions, suggesting a significant role for astroglia in maintaining mitochondrial development and bioenergetics during neuronal differentiation. In an iPSC model of autism spectrum disorder, autism spectrum disorder-derived astrocytes were shown to interfere with normal neuronal development and researchers identified interleukin-6 secretion from these cells as a potential mechanism contributing to autism pathophysiology [Russo et al., 2018]. Models such as these highlight the potential for hiPSC-based coculture systems to investigate the synergistic effects in contributing to both normal functional development in neurons as well as disease onset and progression.

In addition to astrocytes, two other cell type models are being developed for the specific study of interplay between neurons and other central nervous system cell types. Activated hiPSC-derived microglia, cocultured with hiPSC-derived cortical neurons, have been shown to exhibit more physiologically correct behaviors than monoculture controls. These behaviors include the downregulation of pathogen response pathways and a more anti-inflammatory cytokine-based response [Haenseler et al., 2017]. Methods for producing myelinating oligodendrocytes from hiPSC are also available [Wang et al., 2013; Ehrlich et al., 2017]. Such cells have been shown to produce myelin basic protein that engages neurofilament-positive axons from both primary [Wang et al., 2013] and hiPSC-derived [Ehrlich et al., 2017] human neurons when maintained in coculture.

The interaction of neurons with peripheral tissue cell types is also being investigated using hiPSC-based models. Human iPSC-derived motor neurons have been cocultured with skeletal muscle from various human and nonhuman sources as a means to study neuromuscular junction formation and function [Demestre et al., 2015; Puttonen et al., 2015; Santhanam et al., 2018]. Such models can be used to test the efficacy of pharmacological agents as well as to model synaptic breakdown in cases of neuromuscular disorders, such as spinal muscular atrophy [Yoshida et al., 2015]. As our ability to promote skeletal muscle differentiation and maturation from hiPSC sources improves [Chal et al., 2015, 2016; Xi et al., 2017], we can expect further advances in modeling of neuromuscular development, function, and breakdown to occur. Production of hiPSC-derived Schwann cells has been published [Liu et al., 2012; Cai et al., 2017], but their capacity to myelinate human neurons has yet to be reported within in vitro cocultures, highlighting the difficulty in producing such responses in human systems and an important avenue for future study. Innervation of cardiac muscle with sympathetic neurons has been reported and the capacity for neuronal activation to regulate spontaneous beat behavior in hiPSC-derived cardiomyocytes has been demonstrated [Oh et al., 2016]. Reports such as these indicate the potential of hiPSC-derived in vitro coculture systems for studying the interactions between neurons and peripheral tissues. Given that the primary roles of these peripheral neuronal subtypes are to regulate the function of nonneuronal tissues, coculture platforms such as these represent necessary technologies to enable accurate assessment of neuronal function. Not only do such systems represent the means to assay novel drugs and therapeutics, but they also offer the possibility of modeling peripheral neurodegenerative disease states that could not be effectively studied in monocultures of hiPSC-derived neurons.

Movement toward more complex multiorgan models is hampered by the need to maintain optimal culture conditions for disparate cell types. The establishment of 3+ cell or tissue types within controlled in vitro platforms is relatively rare but it is an exciting area of current research due to their potential to model complex tissue-tissue interplay. The development of these so-called organs-on-chips is a rapidly evolving field within bioengineering and is reviewed in detail elsewhere [Huh et al., 2012; Wang et al., 2018b]. The capacity for such systems to model complex neurological function has yet to be demonstrated but remains a tantalizing prospect for future research as our ability to generate more complex models increases.

In addition to multicell type models, movement towards 3-D organoids represents an alternative method for increasing the complexity of hiPSC neuronal models as a means to better recapitulate neurological function in health and disease. Single cell RNA sequencing comparing human cerebral organoids derived from embryonic and hiPSC sources with fetal neocortex has shown that cells differentiated within organoid structures display genetic programs similar to those of the fetal cerebral cortex [Lancaster et al., 2013; Camp et al., 2015]. Specifically, researchers showed that more than 80% of genes implicated in neocortex development that are differentially expressed along the fetal cortex lineage have similar expression profiles in organoid and fetal cerebral cortex samples. This finding is of critical importance as it confirms the ability of such tissue constructs to mirror the development of the

neocortex in humans and therefore its potential to act as a suitable surrogate for studying neurodevelopment. Furthermore, studies of layer specific midbrain markers in neuronal organoids has illustrated that such cells adopt a midbrain dopaminergic phenotype [Tieng et al., 2014; Jo et al., 2016] and express neuromelanin-like granules that are structurally similar to those isolated from human substantia nigra tissues – a feature absent from monolayer cultures and organoids produced from mouse cell sources [Jo et al., 2016]. When using hiPSCs derived from microcephaly patients, researchers found that cells within neuronal organoids exhibited premature neuronal differentiation and highlighted this defect as a potential pathological mechanism in this disease [Lancaster et al., 2013]. Additionally, brain region-specific organoids have been used to model exposure to the Zika virus [Qian et al., 2016]. Infection was shown to increase cell death and reduced proliferation, leading to a decreased neuronal cell layer volume resembling microcephaly. It is clear that advanced organoids have the capacity to obtain results not achievable in simpler 2-D systems and results described herein highlight the value of such culture models in elucidating disease-specific mechanisms.

Future Directions

In recent years, rapid progress has been made in the creation of in vitro systems for studying human neurodevelopment and degeneration. However, significant hurdles remain before the adoption of hiPSC-based systems can offer accurate modeling of human neural tissue. For the most part, both 2-D and 3-D models of the human cortex fail to recapitulate the layered structure of the native tissue. Establishment of such structures is likely to be necessary in order to model higher brain function, including mechanisms related to learning and memory, which may be required for accurate modeling of AD and associated dementias. The use of agarose-alginate gels to create layers of cortical neurons has been shown to offer a viable method for controlling the interaction of layered cortical neurons, although this method has yet to be employed in the maintenance of hiPSC-derived cells [Kunze et al., 2011]. More recently, Kim et al. [2017] used polydimethylsiloxane and collagen hydrogels to recreate the CA3-CA1 hippocampal neural circuit in a monolithic hydrogel and demonstrate functional connectivity between neuronal populations. This work was again achieved using primary rodent neurons and adoption of human cell sources with such bioengineering technologies represents a critical step towards better modeling of human nervous system function. Layered models of hiPSC-derived cortical neurons have been reportedly generated in 3-D using suspension culture and timed application of rostral neutralizing factors to promote the formation of polarized radial glia, intermediate progenitors, and organized, layer-specific cortical neurons [Mariani et al., 2012]. Similarly, Matrigel scaffolds coupled with a spinning bioreactor have been shown to yield cortical-like structures in which the cells were shown to closely follow the endogenous developmental program of the cerebral cortex [Lancaster and Knoblich, 2014]. As such models become more widespread, methods to overcome hypoxia-induced cell death in larger, more cell-dense platforms will become essential. Additionally, protocols for assessing function in these engineered tissues must be carefully considered. Analysis of slices of primary neural tissue are commonly used for electrophysiological assessment and similar methods may be applicable to engineered human tissues. Alternatively, optical mapping or insertion of electrode arrays could be employed to measure function in situ.

For the study of age-related neurodegenerative disease, it is necessary to understand the changes occurring between a healthy, mature neuron and an aged, dysfunctional neuron. Because the study of this phenomenon is challenging in vivo, a hurdle remains to create a comprehensive in vitro model of neurodegeneration that integrates the risks of cellular aging with the positive aspects of hiPSC technology. The work described above regarding the overexpression of progerin to exacerbate age-associated degenerative phenotypes is promising, but the need exists to identify additional molecular drivers of nonsyndromic aging that might predispose human neurons to age-related cellular dysfunction. In this regard, examination of epigenetic factors may represent a plausible target as epigenetic regulation is not only a defined "hallmark of aging" [López-Otín et al., 2013] but also plays a critical role in neuronal function and cognition. Importantly, HDAC inhibitors can ameliorate a broad range of neurodegenerative phenotypes [Hommet et al., 2007; Xuan et al., 2012; Falkenberg and Johnstone, 2014] and hiPSC-derived neurons are amenable to preclinical screening paradigms [Medda et al., 2016; Brownjohn et al., 2017].

The generation of more complex cortical models is likely to have a negative impact on throughput, cost, and potentially culture consistency. While this may be a minor concern to academic laboratories, such considerations may limit the applicability of novel culture platforms for use in preclinical therapeutic development. Consequen-

tially, an issue worthy of discussion is exactly how much complexity is necessary to effectively model a given biological mechanism. It seems sensible that future screening modalities will offer a range of complexities for studying different aspects of neurodegenerative disease. Simple cell-based models can likely be relied on for investigation of basic cellular mechanisms. Multicell type, organized 3-D models may then be employed at later stages of therapy development to assay what impact modulation of these specific mechanisms within a given cell type has on overall function within engineered human neural tissues.

Although cocultures of hiPSC-derived neurons with microglia have been reported [Haenseler et al., 2017], their ability to faithfully recapitulate inflammatory responses within in vitro models has yet to be demonstrated. Neuroinflammation is a hallmark of AD and neurodegenerative diseases in general [Heneka et al., 2015; Walters et al., 2016]. As such, inclusion of inflammatory cell types may well be necessary to induce a clinically relevant phenotype within engineered, hiPSC-based neural tissues. How microglia might be effectively included is an issue that has not been adequately addressed in most in vitro AD research. In addition to microglia, circulating macrophages would be necessary to effectively model neuroinflammation, but methods to create a circulating cell component within neuronal models represents and added layer of complexity that may be difficult to overcome in a reliable, reproducible, and cost-effective manner. This point raises the more general issue of how closely hiPSC-derived neuronal models can mimic the clinical phenotype presented in patients suffering from neurodegenerative conditions. Certainly, hiPSC-derived neurons have be used in novel high-throughput screening efforts, making them potentially useful for rapid preclinical studies [Medda et al., 2016; Brownjohn et al., 2017]. In light of these studies, it remains unclear whether inclusion of all central nervous system cell types in physiologically representative ratios and organizations is necessary to produce data that is truly predictive. If not, can models incorporating single cell sources be used to provide insight into a wide number of patient mutations or are individual patient-based models necessary? Can such systems be realistically established on a timescale likely to be of benefit to patients?

The more widespread availability of CRISPR/Cas9 gene editing may offer a way to avoid reliance on patient-derived cells and instead lead to the establishment of a core group of mutant lines used to provide more general insight into disease etiology. Integration of CRISPR-edited lines along with isogenic controls into more complex models of the human cortex may allow targeted investigation of specific mutations and their impact on normal cortical function. Furthermore, such systems may represent useful testbeds for preclinical analysis of therapeutic efficacy and toxicity without reliance on the generation of individual patient specific lines for each separate study.

As discussed above, the epigenetic signature of reprogrammed cells may represent a significant road block to the accurate in vitro modeling of human neurodegeneration. Interestingly, hiPSC-based cerebral organoids have been compared to fetal tissue and the transcriptomic dynamics of the engineered tissues was shown to faithfully follow the gene expression trajectories of early-to-mid fetal human brains [Luo et al., 2016]. Demethylated regions identified during organoid development overlapped with fetal brain regulatory elements, suggesting that inclusion of hiPSC-derived neurons in more complex 3-D models may offer an alternative method to improve recapitulation of the epigenetic state of cultured cells. However, whether such techniques can be employed to recreate the transcriptomic and epigenetic signature of aged brains has yet to be demonstrated. Methods to recreate an aged neuronal phenotype must likely be considered in addition to the development of more complex cortical models to ensure both the input cell and the engineered niche offer accurate representations of the native tissue in healthy and neurodegenerative states.

Human iPSC technologies offer an unprecedented level of potential to model any cell type and, therefore, disease state. The use of advanced stem cell models combined with a thorough understanding of the model's advantages and restrictions is likely the best choice moving forward, underscoring the need for productive scientific collaboration as the current limitations of this technology are significant but not insurmountable. Additionally, these limitations can also be applied as lessons for modeling other complex conditions, including cardiomyopathies and cancers. As our ability to generate more accurate, subtype-specific cells with correct transcriptomic and epigenomic signatures improves so will our capacity to effectively model disease toward the elucidation of pathological mechanisms and even the establishment of therapeutic applications for patient-derived hiPSCs. The concordant development of more complex and physiologically relevant models of neural tissue offers exciting prospects for our future ability to model neurological form and function.

Disclosure Statement

The authors declare that they have no conflict of interests.

References

Ambasudhan, R., M. Talantova, R. Coleman, X. Yuan, S. Zhu, S.A. Lipton, et al. (2011) Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. Cell Stem Cell *9:* 113–118.

Andrew, S.E., Y.P. Goldberg, B. Kremer, H. Telenius, J. Theilmann, S. Adam, et al. (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet *4:* 398–403.

Ban, J., P. Bonifazi, G. Pinato, F.D. Broccard, L. Studer, V. Torre, et al. (2006) Embryonic stem cell-derived neurons form functional networks in vitro. Stem Cells *25:* 738–749.

Bar-Nur, O., H.A. Russ, S. Efrat, N. Benvenisty (2011) Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell *9:* 17–23.

Becker, J.S., D. Nicetto, K.S. Zaret (2016) H3K9me3-dependent heterochromatin: barrier to cell fate changes. Trends Genet *32:* 29–41.

Bekris, L.M., C.-E. Yu, T.D. Bird, D.W. Tsuang (2010) Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol *23:* 213–227.

Benayoun, B.A., E.A. Pollina, A. Brunet (2015) Epigenetic regulation of ageing: linking environmental inputs to genomic stability. Nat Rev Mol Cell Biol *16:* 593–610.

Berger, S.L., T. Kouzarides, R. Shiekhattar, A. Shilatifard (2009) An operational definition of epigenetics. Genes Dev *23:* 781–783.

Berndt, A., P. Schoenenberger, J. Mattis, K.M. Tye, K. Deisseroth, P. Hegemann, et al. (2011) High-efficiency channel rhodopsins for fast neuronal stimulation at low light levels. Proc Natl Acad Sci USA *108:* 7595–7600.

Berry, B.J., A.S.T. Smith, C.J. Long, C.C. Martin, J.J. Hickman (2018) Physiological Aβ concentrations produce a more biomimetic representation of the Alzheimer's disease phenotype in iPSC derived human neurons. ACS Chem Neurosci *9:* 1693–1701.

Biffi, E., G. Regalia, A. Menegon, G. Ferrigno, A. Pedrocchi (2013) The influence of neuronal density and maturation on network activity of hippocampal cell cultures: a methodological study. PLoS One *8:* e83899.

Bock, C., E. Kiskinis, G. Verstappen, H. Gu, G. Boulting, Z.D. Smith, et al. (2011) Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell *144:* 439–452.

Boekhoorn, K., M. Joels, P.J. Lucassen (2006) Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. Neurobiol Dis *24:* 1–14.

Bourne, J.N., K.M. Harris (2008) Balancing structure and function at hippocampal dendritic spines. Annu Rev Neurosci *31:* 47–67.

Brownjohn, P.W., J. Smith, E. Portelius, L. Serneels, H. Kvartsberg, B. De Strooper, et al. (2017) Phenotypic screening identifies modulators of amyloid precursor protein processing in human stem cell models of Alzheimer's disease. Stem Cell Rep *8:* 870–882.

Cai, S., L. Han, Q. Ao, Y.-S. Chan, D.K.-Y. Shum (2017) Human induced pluripotent cell-derived sensory neurons for fate commitment of bone marrow-derived Schwann cells: implications for remyelination therapy. Stem Cells Transl Med *6:* 369–381.

Camp, J.G., F. Badsha, M. Florio, S. Kanton, T. Gerber, M. Wilsch-Bräuninger, et al. (2015) Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. Proc Natl Acad Sci USA *112:* 15672–15677.

Carson, D., M. Hnilova, X. Yang, C.L. Nemeth, J.H. Tsui, A.S.T. Smith, et al. (2016) Nanotopography-induced structural anisotropy and sarcomere development in human cardiomyocytes derived from induced pluripotent stem cells. ACS Appl Mater Interfaces *8:* 21923–21932.

Chakradhar, S. (2016) An eye to the future: researchers debate best path for stem cell-derived therapies. Nat Med *22:* 116–119.

Chal, J., Z. Al Tanoury, M. Hestin, B. Gobert, S. Aivio, A. Hick, et al. (2016) Generation of human muscle fibers and satellite-like cells from human pluripotent stem cells in vitro. Nat Protoc *11:* 1833–1850.

Chal, J., M. Oginuma, Z. Al Tanoury, B. Gobert, O. Sumara, A. Hick, et al. (2015) Differentiation of pluripotent stem cells to muscle fiber to model Duchenne muscular dystrophy. Nat Biotechnol *33:* 962–969.

Chambers, S.M., C.A. Fasano, E.P. Papapetrou, M. Tomishima, M. Sadelain, L. Studer (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol *27:* 275–280.

Chang, D.T.W., I.J. Reynolds (2006) Differences in mitochondrial movement and morphology in young and mature primary cortical neurons in culture. Neuroscience *141:* 727–736.

Chen, J., H. Liu, J. Liu, J. Qi, B. Wei, J. Yang, et al. (2013) H3K9 methylation is a barrier during somatic cell reprogramming into iPSCs. Nat Genet *45:* 34–42.

Chen, X., K. Zhang, L. Zhou, X. Gao, J. Wang, Y. Yao, et al. (2016) Coupled electrophysiological recording and single cell transcriptome analyses revealed molecular mechanisms underlying neuronal maturation. Protein Cell *7:* 175–186.

Chin, M.H., M. Pellegrini, K. Plath, W.E. Lowry (2010) Molecular analyses of human induced pluripotent stem cells and embryonic stem cells. Cell Stem Cell *7:* 263–269.

Chouliaras, L., D. Mastroeni, E. Delvaux, A. Grover, G. Kenis, P.R. Hof, et al. (2013) Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients. Neurobiol Aging *34:* 2091–2099.

Christopher, M.A., S.M. Kyle, D.J. Katz (2017) Neuroepigenetic mechanisms in disease. Epigenetics Chromatin *10:* 47.

Cuadrado-Tejedor, M., C. Garcia-Barroso, J.A. Sánchez-Arias, O. Rabal, M. Pérez-González, S. Mederos, et al. (2017) A first-in-class small-molecule that acts as a dual inhibitor of HDAC and PDE5 and that rescues hippocampal synaptic impairment in Alzheimer's disease mice. Neuropsychopharmacology *42:* 524–539.

Cullen, D.K., M.E. Gilroy, H.R. Irons, M.C. Laplaca (2010) Synapse-to-neuron ratio is inversely related to neuronal density in mature neuronal cultures. Brain Res *1359:* 44–55.

D'Addario, C., S.B. Candia, B. Arosio, M. Di Bartolomeo, C. Abbate, A. Casè, et al. (2017) Transcriptional and epigenetic phenomena in peripheral blood cells of monozygotic twins discordant for Alzheimer's disease, a case report. J Neurol Sci *372:* 211–216.

Deecke, L., P. Dal-Bianco (1991) Age-Associated Neurological Diseases. New York, Springer.

Demestre, M., M. Orth, K.J. Föhr, K. Achberger, A.C. Ludolph, S. Liebau, et al. (2015) Formation and characterisation of neuromuscular junctions between hiPSC derived motoneurons and myotubes. Stem Cell Res *15:* 328–336.

Devlin, A.-C., K. Burr, S. Borooah, J.D. Foster, E.M. Cleary, I. Geti, et al. (2015) Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. Nat Commun *6:* 5999.

Dodla, M.C., J. Mumaw, S.L. Stice (2010) Role of astrocytes, soluble factors, cells adhesion molecules and neurotrophins in functional synapse formation: implications for human embryonic stem cell derived neurons. Curr Stem Cell Res Ther *5:* 251–260.

Du, F., Q. Yu, A. Chen, D. Chen, S.S. Yan (2018) Astrocytes attenuate mitochondrial dysfunctions in human dopaminergic neurons derived from iPSC. Stem Cell Reports *10:* 366–374.

Eaton, S.L., T.M. Wishart (2017) Bridging the gap: large animal models in neurodegenerative research. Mamm Genome *28:* 324–337.

Ebrahimi, B. (2015) Reprogramming barriers and enhancers: strategies to enhance the efficiency and kinetics of induced pluripotency. Cell Regen *4:* 10.

Ehrlich, M., S. Mozafari, M. Glatza, L. Starost, S. Velychko, A.-L. Hallmann, et al. (2017) Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. Proc Natl Acad Sci USA *114:* E2243–E2252.

Ernst, A., K. Alkass, S. Bernard, M. Salehpour, S. Perl, J. Tisdale, et al. (2014) Neurogenesis in the striatum of the adult human brain. Cell *156:* 1072–1083.
European Academies' Science Advisory Council (2017) Genome editing: scientific opportunities, public interests and policy options in the European Union. https://easac.eu/fileadmin/PDF_s/reports_statements/Genome_Editing/EASAC_Report_31_on_Genome_Editing.pdf.
Falkenberg, K.J., R.W. Johnstone (2014) Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov *13:* 673–691.
Ferrari, A., M. Cecchini, A. Dhawan, S. Micera, I. Tonazzini, R. Stabile, et al. (2011) Nanotopographic control of neuronal polarity. Nano Lett *11:* 505–511.
Ficz, G., M.R. Branco, S. Seisenberger, F. Santos, F. Krueger, T.A. Hore, et al. (2011) Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature *473:* 398–402.
Franz, D., H.L. Olsen, O. Klink, J. Gimsa (2017) Automated and manual patch clamp data of human induced pluripotent stem cell-derived dopaminergic neurons. Sci Data *4:* 170056.
Friedli, M., P. Turelli, A. Kapopoulou, B. Rauwel, N. Castro-Díaz, H.M. Rowe, et al. (2014) Loss of transcriptional control over endogenous retroelements during reprogramming to pluripotency. Genome Res *24:* 1251–1259.
Gaspar-Maia, A., Z.A. Qadeer, D. Hasson, K. Ratnakumar, N.A. Leu, G. Leroy, et al. (2013) MacroH2A histone variants act as a barrier upon reprogramming towards pluripotency. Nat Commun *4:* 1565.
Germain, P.-L., G. Testa (2017) Taming human genetic variability: transcriptomic meta-analysis guides the experimental design and interpretation of iPSC-based disease modeling. Stem Cell Reports *8:* 1784–1796.
Gore, A., Z. Li, H.-L. Fung, J.E. Young, S. Agarwal, J. Antosiewicz-Bourget, et al. (2011) Somatic coding mutations in human induced pluripotent stem cells. Nature *471:* 63–67.
Götz, M., A. Stoykova, P. Gruss (1998) Pax6 controls radial glia differentiation in the cerebral cortex. Neuron *21:* 1031–1044.
Gouder, L., J.-Y. Tinevez, H. Goubran-Botros, A. Benchoua, T. Bourgeron, I. Cloëz-Tayarani (2015) Three-dimensional quantification of dendritic spines from pyramidal neurons derived from human induced pluripotent stem cells. J Vis Exp DOI: 10.3791/53197.
Gräff, J., D. Rei, J.-S. Guan, W.-Y. Wang, J. Seo, K.M. Hennig, et al. (2012) An epigenetic blockade of cognitive functions in the neurodegenerating brain. Nature *483:* 222–226.
Grow, D.A., J.R. McCarrey, C.S. Navara (2016) Advantages of nonhuman primates as preclinical models for evaluating stem cell-based therapies for Parkinson's disease. Stem Cell Res *17:* 352–366.
Gu, X.Q., G.G. Haddad (2018) Maturation of neuronal excitability in hippocampal neurons of mice chronically exposed to cyclic hypoxia. Am J Physiol Cell Physiol *284:* C1156–C1163.
Gunhanlar, N., G. Shpak, M. van der Kroeg, L.A. Gouty-Colomer, S.T. Munshi, B. Lendemeijer, et al. (2018) A simplified protocol for differentiation of electrophysiologically mature neuronal networks from human induced pluripotent stem cells. Mol Psychiatry *23:* 1336–1344.
Haenseler, W., S.N. Sansom, J. Buchrieser, S.E. Newey, C.S. Moore, F.J. Nicholls, et al. (2017) A highly efficient human pluripotent stem cell microglia model displays a neuronal-coculture-specific expression profile and inflammatory response. Stem Cell Reports *8:* 1727–1742.
Hanashima, C., S.C. Li, L. Shen, E. Lai, G. Fishell (2004) Foxg1 suppresses early cortical cell fate. Science *303:* 56–59.
Handel, A.E., S. Chintawar, T. Lalic, E. Whiteley, J. Vowles, A. Giustacchini, et al. (2016) Assessing similarity to primary tissue and cortical layer identity in induced pluripotent stem cell-derived cortical neurons through single-cell transcriptomics. Hum Mol Genet *25:* 989–1000.
Harris, K.M., F.E. Jensen, B. Tsao (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci *12:* 2685–2705.
Hawkins, R.D., G.C. Hon, L.K. Lee, Q. Ngo, R. Lister, M. Pelizzola, et al. (2010) Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. Cell Stem Cell *6:* 479–491.
He, Z., Q. Yu (2018) Identification and characterization of functional modules reflecting transcriptome transition during human neuron maturation. BMC Genomics *19:* 262.
Heneka, M.T., M.J. Carson, J. El Khoury, G.E. Landreth, F. Brosseron, D.L. Feinstein, et al. (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol *14:* 388–405.
Hicks, M.R., J. Hiserodt, K. Paras, W. Fujiwara, A. Eskin, M. Jan, et al. (2018) ERBB3 and NGFR mark a distinct skeletal muscle progenitor cell in human development and hPSCs. Nat Cell Biol *20:* 46–57.
Holland, M.L., R. Lowe, P.W. Caton, C. Gemma, G. Carbajosa, A.F. Danson, et al. (2016) Early-life nutrition modulates the epigenetic state of specific rDNA genetic variants in mice. Science *353:* 495–498.
Hommet, C., K. Mondon, B. De Toffol, T. Constans (2007) Reversible cognitive and neurological symptoms during valproic acid therapy. J Am Geriatr Soc *55:* 628–628.
Hong, S.G., T. Winkler, C. Wu, V. Guo, S. Pittaluga, A. Nicolae, et al. (2014) Path to the clinic: assessment of iPSC-based cell therapies in vivo in a nonhuman primate model. Cell Rep *7:* 1298–1309.
Hu, W., B. Qiu, W. Guan, Q. Wang, M. Wang, W. Li, et al. (2015) Direct conversion of normal and Alzheimer's disease human fibroblasts into neuronal cells by small molecules. Cell Stem Cell *17:* 204–212.
Hu, B.-Y., J.P. Weick, J. Yu, L.-X. Ma, X.-Q. Zhang, J.A. Thomson, et al. (2010) Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc Natl Acad Sci USA *107:* 4335–4340.
Huh, D., Y. Torisawa, G.A. Hamilton, H.J. Kim, D.E. Ingber (2012) Microengineered physiological biomimicry: organs-on-chips. Lab Chip *12:* 2156.
Huh, C.J., B. Zhang, M.B. Victor, S. Dahiya, L.F. Batista, S. Horvath, et al. (2016) Maintenance of age in human neurons generated by microRNA-based neuronal conversion of fibroblasts. Elife *5.*
Hussein, S.M., N.N. Batada, S. Vuoristo, R.W. Ching, R. Autio, E. Närvä, et al. (2011) Copy number variation and selection during reprogramming to pluripotency. Nature *471:* 58–62.
Idda, M.L., R. Munk, K. Abdelmohsen, M. Gorospe (2018) Noncoding RNAs in Alzheimer's disease. Wiley Interdiscip Rev RNA *9:* e1463.
Ihry, R.J., K.A. Worringer, M.R. Salick, E. Frias, D. Ho, K. Theriault, et al. (2018) p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med *24:* 939–946.
Ip, J.P.K., N. Mellios, M. Sur (2018) Rett syndrome: insights into genetic, molecular and circuit mechanisms. Nat Rev Neurosci *19:* 368–382.
Ivanov, A.V., H. Peng, V. Yurchenko, K.L. Yap, D.G. Negorev, D.C. Schultz, et al. (2007) PHD domain-mediated E3 ligase activity directs intramolecular sumoylation of an adjacent bromodomain required for gene silencing. Mol Cell *28:* 823–837.
Jessop, P., M. Toledo-Rodriguez (2018) Hippocampal TET1 and TET2 expression and DNA hydroxymethylation are affected by physical exercise in aged mice. Front Cell Dev Biol *6:* 45.
Jiang, H., Y. Rao (2005) Axon formation: fate versus growth. Nat Neurosci *8:* 544–546.
Jo, J., Y. Xiao, A.X. Sun, E. Cukuroglu, H.-D. Tran, J. Göke, et al. (2016) Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. Cell Stem Cell *19:* 248–257.
Johnson, C., M.O. Warmoes, X. Shen, J.W. Locasale (2015) Epigenetics and cancer metabolism. Cancer Lett *356:* 309–314.
Kang, S., X. Chen, S. Gong, P. Yu, S. Yau, Z. Su, et al. (2017) Characteristic analyses of a neural differentiation model from iPSC-derived neuron according to morphology, physiology, and global gene expression pattern. Sci Rep *7:* 12233.

Karimi, M.M., P. Goyal, I.A. Maksakova, M. Bilenky, D. Leung, J.X. Tang, et al. (2011) DNA methylation and SETDB1/H3K9me3 regulate predominantly distinct sets of genes, retroelements, and chimeric transcripts in mESCs. Cell Stem Cell *8:* 676–687.

Kaufmann, M., A. Schuffenhauer, I. Fruh, J. Klein, A. Thiemeyer, P. Rigo, et al. (2015) High-throughput screening using iPSC-derived neuronal progenitors to identify compounds counteracting epigenetic gene silencing in fragile X syndrome. J Biomol Screen *20:* 1101–1111.

Kaur, P., D.S. Karolina, S. Sepramaniam, A. Armugam, K. Jeyaseelan (2014) Expression profiling of RNA transcripts during neuronal maturation and ischemic injury. PLoS One *9:* e103525.

Kayama, T., I. Suzuki, A. Odawara, T. Sasaki, Y. Ikegaya (2018) Temporally coordinated spiking activity of human induced pluripotent stem cell-derived neurons co-cultured with astrocytes. Biochem Biophys Res Commun *495:* 1028–1033.

Kilpinen, H., A. Goncalves, A. Leha, V. Afzal, K. Alasoo, S. Ashford, et al. (2017) Common genetic variation drives molecular heterogeneity in human iPSCs. Nature *546:* 370–375.

Kim, K., A. Doi, B. Wen, K. Ng, R. Zhao, P. Cahan, et al. (2010): Epigenetic memory in induced pluripotent stem cells. Nature *467:* 285–290.

Kim, S.H., S.-K. Im, S.-J. Oh, S. Jeong, E.-S. Yoon, C.J. Lee, et al. (2017) Anisotropically organized three-dimensional culture platform for reconstruction of a hippocampal neural network. Nat Commun *8:* 14346.

Kolanowski, T.J., C.L. Antos, K. Guan (2017) Making human cardiomyocytes up to date: derivation, maturation state and perspectives. Int J Cardiol *241:* 379–386.

Kong, G., Z. Huang, W. Ji, X. Wang, J. Liu, X. Wu, et al. (2017) The ketone metabolite β-hydroxybutyrate attenuates oxidative stress in spinal cord injury by suppression of class I histone deacetylases. J Neurotrauma *34:* 2645–2655.

Konur S, Rabinowitz D, Fenstermaker VL, Yuste R: Systematic regulation of spine sizes and densities in pyramidal neurons. J Neurobiol 2003;56:95–112.

Kubben, N., T. Misteli (2017) Shared molecular and cellular mechanisms of premature ageing and ageing-associated diseases. Nat Rev Mol Cell Biol *18:* 595–609.

Kuijlaars, J., T. Oyelami, A. Diels, J. Rohrbacher, S. Versweyveld, G. Meneghello, et al. (2016) Sustained synchronized neuronal network activity in a human astrocyte co-culture system. Sci Rep *6:* 36529.

Kunze, A., M. Giugliano, A. Valero, P. Renaud (2011) Micropatterning neural cell cultures in 3D with a multi-layered scaffold. Biomaterials *32:* 2088–2098.

Kyttälä, A., R. Moraghebi, C. Valensisi, J. Kettunen, C. Andrus, K.K. Pasumarthy, et al. (2016) Genetic variability overrides the impact of parental cell type and determines iPSC differentiation potential. Stem Cell Reports *6:* 200–212.

Lam, R.S., F.M. Töpfer, P.G. Wood, V. Busskamp, E. Bamberg (2017) Functional maturation of human stem cell-derived neurons in long-term cultures. PLoS One *12:* e0169506.

Lancaster, M.A., J.A. Knoblich (2014) Generation of cerebral organoids from human pluripotent stem cells. Nat Protoc *9:* 2329–2340.

Lancaster, M.A., M. Renner, C.-A. Martin, D. Wenzel, L.S. Bicknell, M.E. Hurles, et al. (2013) Cerebral organoids model human brain development and microcephaly. Nature *501:* 373–379.

Laurent, L.C., I. Ulitsky, I. Slavin, H. Tran, A. Schork, R. Morey, et al. (2011) Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell *8:* 106–118.

Lavratti, C., G. Dorneles, D. Pochmann, A. Peres, A. Bard, L. de Lima Schipper, et al. (2017) Exercise-induced modulation of histone H4 acetylation status and cytokines levels in patients with schizophrenia. Physiol Behav *168:* 84–90.

Lewis, T.L., G.F. Turi, S.-K. Kwon, A. Losonczy, F. Polleux (2016) Progressive decrease of mitochondrial motility during maturation of cortical axons in vitro and in vivo. Curr Biol *26:* 2602–2608.

Li, P., L. Wang, B.D. Bennett, J. Wang, J. Li, Y. Qin, et al. (2017) Rif1 promotes a repressive chromatin state to safeguard against endogenous retrovirus activation. Nucleic Acids Res *45:* 12723–12738.

Li, B., H. Yamamori, Y. Tatebayashi, B. Shafit-Zagardo, H. Tanimukai, S. Chen, et al. (2008) Failure of neuronal maturation in Alzheimer disease dentate gyrus. J Neuropathol Exp Neurol *67:* 78–84.

Lin, Y.-T., J. Seo, F. Gao, H.M. Feldman, H.-L. Wen, J. Penney, et al. (2018) APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. Neuron *98:* 1141–1154.e7.

Lin, M.-Y., Z.-H. Sheng (2015) Regulation of mitochondrial transport in neurons. Exp Cell Res *334:* 35–44.

Liu, Q., S.C. Spusta, R. Mi, R.N.T. Lassiter, M.R. Stark, A. Höke, et al. (2012) Human neural crest stem cells derived from human ESCs and induced pluripotent stem cells: induction, maintenance, and differentiation into functional Schwann cells. Stem Cells Transl Med *1:* 266–278.

López-Otín, C., M.A. Blasco, L. Partridge, M. Serrano, G. Kroemer (2013) The hallmarks of aging. Cell *153:* 1194–1217.

Lopizzo, N., L. Bocchio Chiavetto, N. Cattane, G. Plazzotta, F.I. Tarazi, C.M. Pariante, et al. (2015) Gene-environment interaction in major depression: focus on experience-dependent biological systems. Front Psychiatry *6:* 68.

Lovat, V., D. Pantarotto, L. Lagostena, B. Cacciari, M. Grandolfo, M. Righi, et al. (2005) Carbon nanotube substrates boost neuronal electrical signaling *5:* 1107–1110.

Lundy, S.D., W.-Z. Zhu, M. Regnier, M.A. Laflamme (2013) Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Stem Cells Dev *22:* 1991–2002.

Luo, C., M.A. Lancaster, R. Castanon, J.R. Nery, J.A. Knoblich, J.R. Ecker (2016) Cerebral organoids recapitulate epigenomic signatures of the human fetal brain. Cell Rep *17:* 3369–3384.

Maksakova, I.A., P. Goyal, J. Bullwinkel, J.P. Brown, M. Bilenky, D.L. Mager, et al. (2011) H3K9me3-binding proteins are dispensable for SETDB1/H3K9me3-dependent retroviral silencing. Epigenetics Chromatin *4:* 12.

Maoz, R., B.P. Garfinkel, H. Soreq (2017) Alzheimer's Disease and ncRNAs. Cham, Springer, pp 337–361.

Mariani, J., M.V. Simonini, D. Palejev, L. Tomasini, G. Coppola, A.M. Szekely, et al. (2012) Modeling human cortical development in vitro using induced pluripotent stem cells. Proc Natl Acad Sci USA *109:* 12770–12775.

Martinez-Barbera, J.P., M. Signore, P.P. Boyl, E. Puelles, D. Acampora, R. Gogoi, et al. (2001) Regionalisation of anterior neuroectoderm and its competence in responding to forebrain and midbrain inducing activities depend on mutual antagonism between OTX2 and GBX2. Development *128:* 4789–4800.

Martins-Taylor, K., B.S. Nisler, S.M. Taapken, T. Compton, L. Crandall, K.D. Montgomery, et al. (2011) Recurrent copy number variations in human induced pluripotent stem cells. Nat Biotechnol *29:* 488–491.

Matsui, T., D. Leung, H. Miyashita, I.A. Maksakova, H. Miyachi, H. Kimura, et al. (2010) Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature *464:* 927–931.

Medda, X., L. Mertens, S. Versweyveld, A. Diels, L. Barnham, A. Bretteville, et al. (2016) Development of a scalable, high-throughput-compatible assay to detect tau aggregates using iPSC-derived cortical neurons maintained in a three-dimensional culture format. J Biomol Screen *21:* 804–815.

Mennerick, S., M. Chisari, H.-J. Shu, A. Taylor, M. Vasek, L.N. Eisenman, et al. (2010) Diverse voltage-sensitive dyes modulate GABAA receptor function. J Neurosci *30:* 2871–2879.

Mertens, J., M.C. Marchetto, C. Bardy, F.H. Gage (2016) Evaluating cell reprogramming, differentiation and conversion technologies in neuroscience. Nat Rev Neurosci *17:* 424–437.

Mertens, J., A.C.M. Paquola, M. Ku, E. Hatch, L. Böhnke, S. Ladjevardi, et al. (2015) Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. Cell Stem Cell *17:* 705–718.

Miles, D.C., N.A. de Vries, S. Gisler, C. Lieftink, W. Akhtar, E. Gogola, et al. (2017) TRIM28 Is an epigenetic barrier to induced pluripotent stem cell reprogramming. Stem Cells *35:* 147–157.

Millan, M.J. (2017) Linking deregulation of non-coding RNA to the core pathophysiology of Alzheimer's disease: an integrative review. Prog Neurobiol *156:* 1–68.

Miller, J.D., Y.M. Ganat, S. Kishinevsky, R.L. Bowman, B. Liu, E.Y. Tu, et al. (2013) Human iPSC-based modeling of late-onset disease via progerin-induced aging. Cell Stem Cell *13:* 691–705.

Miller, J.C., M.C. Holmes, J. Wang, D.Y. Guschin, Y.-L. Lee, I. Rupniewski, et al. (2007) An improved zinc-finger nuclease architecture for highly specific genome editing. Nat Biotechnol *25:* 778–785.

National Institutes of Health: Somatic cell genome editing. 2018. https://commonfund.nih.gov/editing.

Nazor, K.L., G. Altun, C. Lynch, H. Tran, J.V. Harness, I. Slavin, et al. (2012) Recurrent variations in DNA methylation in human pluripotent stem cells and their differentiated derivatives. Cell Stem Cell *10:* 620–634.

Nishimura, K., M. Sano, M. Ohtaka, B. Furuta, Y. Umemura, Y. Nakajima, et al. (2011) Development of defective and persistent Sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming. J Biol Chem *286:* 4760–4771.

Nishino, K., M. Toyoda, M. Yamazaki-Inoue, Y. Fukawatase, E. Chikazawa, H. Sakaguchi, et al. (2011) DNA methylation dynamics in human induced pluripotent stem cells over time. PLoS Genet *7:* e1002085.

Obien, M.E.J., K. Deligkaris, T. Bullmann, D.J. Bakkum, U. Frey (2014) Revealing neuronal function through microelectrode array recordings. Front Neurosci *8:* 423.

Odawara, A., H. Katoh, N. Matsuda, I. Suzuki (2016) Physiological maturation and drug responses of human induced pluripotent stem cell-derived cortical neuronal networks in long-term culture. Sci Rep *6:* 26181.

Odawara, A., Y. Saitoh, A.H. Alhebshi, M. Gotoh, I. Suzuki (2014) Long-term electrophysiological activity and pharmacological response of a human induced pluripotent stem cell-derived neuron and astrocyte co-culture. Biochem Biophys Res Commun *443:* 1176—1181.

Oh, Y., G.-S. Cho, Z. Li, I. Hong, R. Zhu, M.-J. Kim, et al. (2016) Functional coupling with cardiac muscle promotes maturation of hPSC-derived sympathetic neurons. Cell Stem Cell *19:* 95–106.

Ohnuki, M., K. Tanabe, K. Sutou, I. Teramoto, Y. Sawamura, M. Narita, et al. (2014) Dynamic regulation of human endogenous retroviruses mediates factor-induced reprogramming and differentiation potential. Proc Natl Acad Sci USA *111:* 12426–12431.

Penn, Y., M. Segal, E. Moses (2016) Network synchronization in hippocampal neurons. Proc Natl Acad Sci USA *113:* 3341–3346.

Pevny, L.H., S. Sockanathan, M. Placzek, R. Lovell-Badge (1998) A role for SOX1 in neural determination. Development *125:* 1967–1978.

Polo, J.M., S. Liu, M.E. Figueroa, W. Kulalert, S. Eminli, K.Y. Tan, et al. (2010) Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat Biotechnol *28:* 848–855.

Prè, D., M.W. Nestor, A.A. Sproul, S. Jacob, P. Koppensteiner, V. Chinchalongporn, et al. (2014) A time course analysis of the electrophysiological properties of neurons differentiated from human induced pluripotent stem cells (iPSCs). PLoS One *9:* e103418.

Puttonen, K.A., M. Ruponen, N. Naumenko, O.H. Hovatta, P. Tavi, J. Koistinaho (2015) Generation of functional neuromuscular junctions from human pluripotent stem cell lines. Front Cell Neurosci *9:* 473.

Qian, X., H.N. Nguyen, M.M. Song, C. Hadiono, S.C. Ogden, C. Hammack, et al. (2016) Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell *165:* 1238–1254.

Robinton, D.A., G.Q. Daley (2012) The promise of induced pluripotent stem cells in research and therapy. Nature *481:* 295–305.

Roessler, R., S.A. Smallwood, J.V. Veenvliet, P. Pechlivanoglou, S.-P. Peng, K. Chakrabarty, et al. (2014) Detailed analysis of the genetic and epigenetic signatures of iPSC-derived mesodiencephalic dopaminergic neurons. Stem Cell Reports *2:* 520–533.

Roubroeks, J.A.Y., R.G. Smith, D.L.A. van den Hove, K. Lunnon (2017) Epigenetics and DNA methylomic profiling in Alzheimer's disease and other neurodegenerative diseases. J Neurochem *143:* 158–170.

Rouhani, F., N. Kumasaka, M.C. de Brito, A. Bradley, L. Vallier, D. Gaffney (2014) Genetic background drives transcriptional variation in human induced pluripotent stem cells. PLoS Genet *10:* e1004432.

Russo, F.B., B.C. Freitas, G.C. Pignatari, I.R. Fernandes, J. Sebat, A.R. Muotri, et al. (2018) Modeling the interplay between neurons and astrocytes in autism using human induced pluripotent stem cells. Biol Psychiatry *83:* 569–578.

Ryan, N.S., M.N. Rossor (2010) Correlating familial Alzheimer's disease gene mutations with clinical phenotype. Biomark Med *4:* 99–112.

Sanjana, N.E., O. Shalem, F. Zhang (2014) Improved vectors and genome-wide libraries for CRISPR screening. Nat Methods *11:* 783–784.

Santhanam, N., L. Kumanchik, X. Guo, F. Sommerhage, Y. Cai, M. Jackson, et al. (2018) Stem cell derived phenotypic human neuromuscular junction model for dose response evaluation of therapeutics. Biomaterials *166:* 64–78.

Schaarschmidt, G., S. Schewtschik, R. Kraft, F. Wegner, J. Eilers, J. Schwarz, et al. (2009) A new culturing strategy improves functional neuronal development of human neural progenitor cells. J Neurochem *109:* 238–247.

Schlaeger, T.M., L. Daheron, T.R. Brickler, S. Entwisle, K. Chan, A. Cianci, et al. (2015) A comparison of non-integrating reprogramming methods. Nat Biotechnol *33:* 58–63.

Selvaraj, V., P. Jiang, O. Chechneva, U.-G. Lo, W. Deng (2012) Differentiating human stem cells into neurons and glial cells for neural repair. Front Biosci (Landmark Ed) *17:* 65–89.

Shahbazi, E., F. Mirakhori, V. Ezzatizadeh, H. Baharvand (2018) Reprogramming of somatic cells to induced neural stem cells. Methods *133:* 21–28.

Sheehy, S.P., F. Pasqualini, A. Grosberg, S.J. Park, Y. Aratyn-Schaus, K.K. Parker (2014) Quality metrics for stem cell-derived cardiac myocytes. Stem Cell Reports *2:* 282–94.

Shimazu, T., M.D. Hirschey, J. Newman, W. He, K. Shirakawa, N. Le Moan, et al. (2013) Suppression of oxidative stress by hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science *339:* 211–214.

Song, L., K. Wang, Y. Li, Y. Yang (2016) Nanotopography promoted neuronal differentiation of human induced pluripotent stem cells. Colloids Surf B Biointerfaces *148:* 49–58.

Spalding, K.L., O. Bergmann, K. Alkass, S. Bernard, M. Salehpour, H.B. Huttner, et al. (2013) Dynamics of hippocampal neurogenesis in adult humans. Cell *153:* 1219–1227.

Studer, L., E. Vera, D. Cornacchia (2015) Programming and reprogramming cellular age in the era of induced pluripotency. Cell Stem Cell *16:* 591–600.

Sun, Z., D.J. Williams, B. Xu, J.A. Gogos (2018) Altered function and maturation of primary cortical neurons from a 22q11.2 deletion mouse model of schizophrenia. Transl Psychiatry *8:* 85.

Takahashi, K., S. Yamanaka (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126:* 663–676.

Takazawa, T., G.F. Croft, M.W. Amoroso, L. Studer, H. Wichterle, A.B. MacDermott (2012) Maturation of spinal motor neurons derived from human embryonic stem cells. PLoS One *7:* e40154.

Tang, M., Q. Song, N. Li, Z. Jiang, R. Huang, G. Cheng (2013) Enhancement of electrical signaling in neural networks on graphene films. Biomaterials *34:* 6402–6411.

Tao, H., P. Xie, Y. Cao, L. Shu, L. Li, J. Chen, et al. (2018) The dynamic DNA demethylation during postnatal neuronal development and neural stem cell differentiation. Stem Cells Int *2018:* 1–10.

Tieng, V., L. Stoppini, S. Villy, M. Fathi, M. Dubois-Dauphin, K.-H. Krause (2014) Engineering of midbrain organoids containing long-lived dopaminergic neurons. Stem Cells Dev *23:* 1535–1547.
Toda, T., S.L. Parylak, S.B. Linker, F.H. Gage (2018) The role of adult hippocampal neurogenesis in brain health and disease. Mol Psychiatry DOI: 10.1038/s41380-018-0036-2.
Toh, T.B., J.J. Lim, E.K.-H. Chow (2017) Epigenetics in cancer stem cells. Mol Cancer *16:* 29.
Tsai, S.Q., N. Wyvekens, C. Khayter, J.A. Foden, V. Thapar, D. Reyon, et al. (2014) Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. Nat Biotechnol *32:* 569–576.
Vera, E., N. Bosco, L. Studer (2016) Generating late-onset human iPSC-based disease models by inducing neuronal age-related phenotypes through telomerase manipulation. Cell Rep *17:* 1184–1192.
Volmar, C.-H., H. Salah-Uddin, K.J. Janczura, P. Halley, G. Lambert, A. Wodrich, et al. (2018) Multipronged HDAC strategy for Alzheimer's disease. Biol Psychiatry *83:* S46.
Wainger, B.J., E. Kiskinis, C. Mellin, O. Wiskow, S.S.W. Han, J. Sandoe, et al. (2014) Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep *7:* 1–11.
Walters, A., E. Phillips, R. Zheng, M. Biju, T. Kuruvilla (2016) Evidence for neuroinflammation in Alzheimer's disease. Prog Neurol Psychiatry *20:* 25–31.
Wang, S., J. Bates, X. Li, S. Schanz, D. Chandler-Militello, C. Levine, et al. (2013) Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. Cell Stem Cell *12:* 252–264.
Wang, Y.I., C. Carmona, J.J. Hickman, M.L. Shuler (2018b) Multiorgan microphysiological systems for drug development: strategies, advances, and challenges. Adv Healthc Mater *7:* 1701000.
Wang, C., R. Najm, Q. Xu, D.-E. Jeong, D. Walker, M.E. Balestra, et al. (2018a) Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. Nat Med *24:* 647–657.
Weissbein, U., O. Plotnik, D. Vershkov, N. Benvenisty (2017) Culture-induced recurrent epigenetic aberrations in human pluripotent stem cells. PLoS Genet *13:* e1006979.
Wiggin, G.R., J.P. Fawcett, T. Pawson (2005) Polarity proteins in axon specification and synaptogenesis. Dev Cell *8:* 803–816.
Xi, H., W. Fujiwara, K. Gonzalez, M. Jan, S. Liebscher, B. Van Handel, et al. (2017) In vivo human somitogenesis guides somite development from hPSCs. Cell Rep *18:* 1573–1585.
Xuan, A., D. Long, J. Li, W. Ji, L. Hong, M. Zhang, et al. (2012) Neuroprotective effects of valproic acid following transient global ischemia in rats. Life Sci *90:* 463–468.
Yamamoto, H., T. Demura, M. Morita, G.A. Banker, T. Tanii, S. Nakamura (2012) Differential neurite outgrowth is required for axon specification by cultured hippocampal neurons. J Neurochem *123:* 904–910.
Yang, H.S., N. Ieronimakis, J.H. Tsui, H.N. Kim, K.-Y. Suh, M. Reyes, et al. (2014a) Nanopatterned muscle cell patches for enhanced myogenesis and dystrophin expression in a mouse model of muscular dystrophy. Biomaterials *35:* 1478–1486.
Yang, X., L. Pabon, C.E. Murry (2014b) Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. Circ Res *114:* 511–523.
Yang, S., R. Zhang, G. Wang, Y. Zhang (2017) The development prospection of HDAC inhibitors as a potential therapeutic direction in Alzheimer's disease. Transl Neurodegener *6:* 19.
Yoo, J., M. Noh, H. Kim, N.L. Jeon, B.-S. Kim, J. Kim (2015) Nanogrooved substrate promotes direct lineage reprogramming of fibroblasts to functional induced dopaminergic neurons. Biomaterials *45:* 36–45.
Yoshida, M., S. Kitaoka, N. Egawa, M. Yamane, R. Ikeda, K. Tsukita, et al. (2015) Modeling the early phenotype at the neuromuscular junction of spinal muscular atrophy using patient-derived iPSCs. Stem Cell Reports *4:* 561–568.
Zhou, Y., F. Zeng (2013) Integration-free methods for generating induced pluripotent stem cells. Genomics Proteomics Bioinformatics *11:* 284–287.
Zhu, Q., R. Stöger, R. Alberio (2018) A lexicon of DNA modifications: their roles in embryo development and the germline. Front Cell Dev Biol *6:* 24.
Zhu, W.-Z., B. Van Biber, M.A. Laflamme (2011) Methods for the derivation and use of cardiomyocytes from human pluripotent stem cells. Methods Mol Biol *767:* 419–431.
Ziller, M.J., R. Edri, Y. Yaffe, J. Donaghey, R. Pop, W. Mallard, et al. (2015) Dissecting neural differentiation regulatory networks through epigenetic footprinting. Nature *518:* 355–359.