

Induced pluripotent stem cells (iPSCs) and neurological disease modeling: progress and promises

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Received July 28, 2011; Revised and Accepted July 30, 2011

The systematic generation of neurons from patients with neurological disorders can provide important insights into disease pathology, progression and mechanism. This review will discuss recent progress in modeling neurodegenerative and neurodevelopmental diseases using induced pluripotent stem cells (iPSCs) and highlight some of the current challenges in the field. Combined with other technologies previously used to study brain disease, iPSC modeling has the promise to influence modern medicine on several fronts: early diagnosis, drug development and effective treatment.

INTRODUCTION

To date, most of the studies of human brain and neuronal function in neurological patients have been performed on post-mortem tissues that were not always well preserved and often represented the end-stage of the disease. In addition, mouse models available to study neurological diseases are limited and usually do not fully recapitulate the human neural phenotype. The advent of induced pluripotent stem cells (iPSCs) provided an important tool for the study of human neurodegenerative and neurodevelopmental diseases in live neurons in a controlled environment (1,2). Researchers are just beginning to grasp the many implications of studying developing neurons from patients. For example, reprogramming cells from patients with neurological diseases allows the study of molecular pathways particular to specific subtypes of neurons [e.g. dopaminergic neurons in Parkinson's disease (PD)]; such an experiment can only be done using neurons differentiated from iPSCs, as it is too invasive to isolate these neurons from patients' brains. In addition, because reprogramming technology allows for the study of human neurons during development, disease-specific pathways can be investigated prior to and during disease onset. Detecting disease-specific molecular signatures in live human neurons, as opposed to late-stage postmortem tissues, opens possibilities for early intervention therapies and new diagnostic tools. Importantly, it is now feasible to obtain neurons that capture the genetic material from the patient, which includes not only the

mutated gene(s)—when the gene is known—but also all the genetic modifiers that play an important but yet largely unknown role in the pathology of neurological disease. Lastly, once the neurological neural phenotype is detected *in vitro*, the so-called 'disease-in-a-dish' approach allows for the screening of drugs that can ameliorate the disease-specific phenotype (Fig. 1). New therapeutical drugs could either act on generalized pathways in all patients or be patient-specific and used in a personalized medicine approach.

In this review, we will assess the recent literature on modeling neurological disease using iPSCs (Table 1), propose other neurological diseases that have not yet been explored with iPSC technology and discuss some of the main challenges in the field.

iPSCs FOR NEUROLOGICAL DISEASES

Neurodegenerative diseases

Neurodegenerative disorders include a variety of hereditary or sporadic diseases that involve the chronic, progressive loss of neuronal structure and function. Since aging is the most consistent risk factor for neurodegenerative disease, and we have an aging population, it is of great importance that we unravel the causes of cell death that are characteristic of these diseases. Reprogramming technology allows researchers to study the development and progression of neurodegeneration in a human

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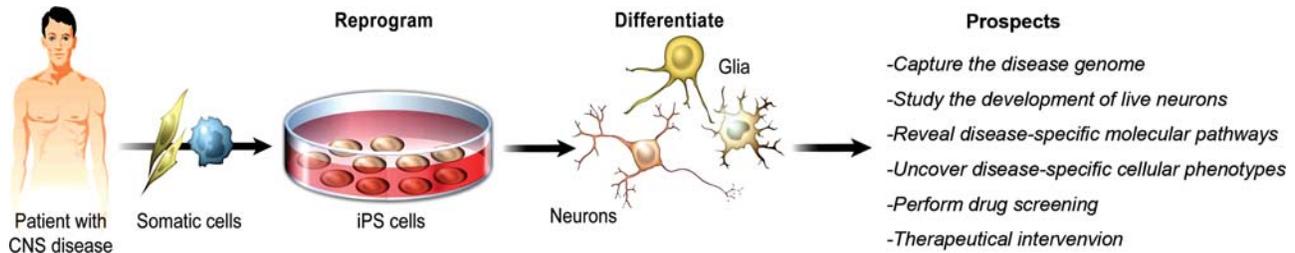


Figure 1. Disease-in-a-dish: using iPSC to model neurological diseases using patients' somatic cells (e.g. skin). Neural progenitors can be generated from iPSC and then give rise to glial cells (oligodendrocytes and astrocytes) and to subtypes of neurons that are relevant for different neurological diseases. Highlighted on this figure are the prospects of using iPSC technology to model for neurological diseases.

system and may enable the discovery of new early diagnostics and therapies.

The first neurodegenerative diseases modeled using human iPSCs were monogenetically inherited, rare and fatal disorders: smooth muscle atrophy (SMA) and familial dysautonomia (FD). To model SMA, iPSCs were generated from a child with a mutation in *SMN* (SMA type 1) and from his unaffected mother (3). Both fibroblasts and iPSCs from the child showed reduced levels of full-length SMN, and motor neurons derived from these cells were unable to survive in culture past 6 weeks. Two compounds known to increase SMN levels, valproic acid and tobramycin, could partially restore the reduction in the SMN protein, though their effects on neuronal survival were not investigated. FD was modeled with three patients and two controls (4). iPSC-derived peripheral neurons, the neuronal subtype primarily affected by this disorder, showed tissue-specific mis-splicing of *IKBKAP*, the transcript of the IKAP protein implicated in FD. Incomplete differentiation and reduced mobility, known results of IKAP depletion, were also observed in FD patient iPSC-derived cells. In addition to providing large-scale transcriptional analysis, this study also showed a partial rescue of the splicing phenotype using kinetin, a compound known to prevent mutant splicing. These studies, while limited, have laid the groundwork for using reprogramming technology to model more complex, multigenic or even idiopathic neurodegenerative disorders.

PD is the most prevalent neurodegenerative movement disorder, commonly characterized by deficits in initiation and control of motor skills. These deficits are traditionally attributed to the death of dopaminergic neurons in the substantia nigra and subsequent loss of dopamine in the striatum. Many mouse models and postmortem tissue studies have provided insight into the pathogenesis of PD; however, the former consistently fail to recapitulate the cardinal features of PD and the latter are end-stage representations. The generation of iPSCs from PD patients has been reported in abstracts and publications, though the focus has been largely methodological (5–7). Nguyen *et al.* described the first biologically relevant cellular phenotype from iPSC-derived neurons from Parkinson's patients. In this study, iPSCs were derived from one PD patient carrying a point mutation in *LRRK2*, the most common PD-related mutation; these iPSCs were differentiated into dopaminergic neurons in parallel with iPSCs from one control patient (8). The study found that dopaminergic neurons from the PD patient expressed increased levels of α -synuclein, the protein whose dysfunction unites all PD cases, and showed

increased sensitivity to cellular stressors including hydrogen peroxide, MG-132 and 6-hydroxydopamine. However, inhibiting LRRK2 activity with a kinase inhibitor did not prevent this phenotype. A slightly larger study, including three patients with mutations in mitochondrial protein PINK1, reported that neurons from patients with PD had decreased mitochondrial recruitment of lentivirally expressed PARKIN; this phenotype was rescued by the forced expression of wild-type PINK1 (9). Further studies, with larger cohorts, will be necessary to confirm the phenotype observed and characterize downstream, potentially therapeutic, molecular targets.

Alzheimer's disease (AD) is the most common neurodegenerative disease, characterized by a severe, progressive dementia. Neuropathology consists of neurofibrillary plaques and tangles composed primarily of amyloid- β ($A\beta$) peptide and hyperphosphorylated TAU, respectively, in the cerebral cortex and some subcortical regions including the hippocampus. Similar to PD, before reprogramming technology, the study of AD was severely limited due to the lack of relevant mouse models. The generation of iPSCs from patients with sporadic forms of the disease, which represent the majority of cases, should provide insight into forms of neurodegeneration that were previously impossible to model. Aside from proof of concept experiments showing that it is possible to reprogram cells from AD patients, the field has been surprisingly slow in the analysis of AD iPSC models, which might reflect the challenges of modeling late-onset diseases (6). The effects of the $A\beta$ peptide on neurogenesis have been inconclusive, but a recent report suggests that treatment with $A\beta_{1-42}$ oligomers may impair the function of human embryonic stem cell (ESC)-derived cholinergic forebrain neurons (10). It remains to be seen whether similar effects will be observed in neurons derived from AD patient iPSCs.

Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) is the most common adult-onset motor neuron disease, mainly characterized by muscular atrophy and weakness accompanied by a fast and progressive degeneration of motor neurons in the cortex, brainstem and spinal cord. Life expectancy is usually 2–5 years after disease onset and there is currently no cure or effective therapy. Clinical trials based on ALS mouse models have largely failed, suggesting a need for the exploration of new ALS models. Two groups have managed to generate iPSCs from two different familial forms (with previously identified mutations). More than 10 different genes have been implicated in ALS, including superoxide dismutase 1 (*SOD1*, ALS1) (11) and vamp-associated protein B/C (*VAPB*, ALS8) (12). Dimos *et al.* (13) generated

Table 1. Summary of iPSC lines generated from patients with neurological diseases

Disease	Genetic mutation	Primary cells	Reprogramming method	Neural differentiation	Relevant phenotype	Reference
PD	Sporadic	Fibroblasts: Coriell, G20446	Retrovirus; 4 factor	No	No	Park (6)
	Sporadic	Fibroblasts: Coriell, G20442, G20443, G20445, G20446, G08395	Lentivirus; excisable, inducible 3/4 factor	Yes (~5% TH+)	No	Soldner (5)
	LRRK2 (G2019S)	Fibroblasts	Retrovirus; 3 factor	Yes (3–5% TH)	Yes: Elevated alpha-synuclein expression, increased sensitivity to cellular stressors	Nguyen (8)
	PINK1 (C1366T, T509G) SNCA (A53T)	Fibroblasts Fibroblasts	Retrovirus; 4 factor Lentivirus; excisable, inducible 4 factor	Yes (10–15% TH of TUJ1) Yes	Yes: Less recruitment of Parkin to the mitochondria No	Seibler (9) Soldner (68) (2011)
ALS	SOD1 (L144F) VAPB (C166T)	Fibroblasts Fibroblasts	Retrovirus, 4 factor Retrovirus, 4 factor	Yes (20% HB9) Yes (5% HB9)	No Yes: Reduced VAPB levels in ALS8 patients	Dimos (13) Mitne-Neto (14)
	SMA	SMN	Fibroblasts	Retrovirus, 4 factor	Yes (~10% CHAT of TUJ1)	Yes: Reduced levels of SMN protein and impaired survival of motor neurons
FD	IKBKAP	Fibroblasts	Lentivirus, 4 factor	Yes (defects in neural crest differentiation)	Yes: Tissue-specific mis-splicing incomplete differentiation, reduced motility	Lee (4)
RTT	MeCP2 (1155del32, Q244X, T158M and R306C)	Fibroblasts: Coriell, GM11272, GM16548, GM17880, GM11270	Retrovirus; 4 factor	Yes	Yes: Fewer synapses, reduced spine density, smaller soma size, altered calcium signaling and electrophysiological defects	Marchetto (27)
	MeCP2 (Δ 3–4, T158M, R306C)	Fibroblasts: Patient biopsy and Coriell, GM17880, GM11270	Retrovirus; 4 factor	Yes	Yes: Smaller soma size	Cheung (24)
FXS	FMR1	Fibroblasts: Coriell, GM05848, GM07072, GM09497	Retrovirus; 4 factor	No	No	Urbach (32)
SCZD	DISC1	Fibroblasts	Episomes; 4 factors	No	No	Chiang (34)
	Sporadic	Fibroblasts: Coriell, GM02038, GM01792, GM01835, GM02497	Tetracycline-inducible lentivirus; 5 factors	Yes	Yes: Decreased neuronal connectivity, neurite number, PSD95-protein levels and glutamate receptor expression	Brennan (35)

ALS, amyotrophic lateral sclerosis; FD, familial dysautonomia; FXS, fragile X; PD, Parkinson's disease; RTT, Rett syndrome; SMA, spinal muscular atrophy; SCZD, schizophrenia.

iPSCs from two octogenarian sisters with mutations in the *SOD1* gene (*SOD1*^{L144F}). Motor neurons were generated from one of the ALS patients, but they were never assayed for a phenotype or compared with neurons from unaffected patients. Mitne-Neto *et al.* (14) reported iPSC lines from four patients with mutations on *VAPB* gene as well as from three unaffected siblings as controls. They detected a significant reduction in the levels of VAPB protein, particularly in a motor neuron-enriched population, suggesting that the reduction in VAPB could be involved in the initial steps of ALS degeneration (14). These observations may be relevant to other forms of ALS, as the reduction in VAPB protein was recently reported in sporadic ALS patients with no identified genetic mutations (15). Because other cells belonging to the motor

neuron niche (i.e., astrocytes and microglia) have been shown to play a role in the pathology of ALS, it remains to be seen if the iPSC-derived cells can also recapitulate the non-cell autonomous aspects of the disease (16–19).

Neurodevelopmental disorders

Neurodevelopmental disorders include a wide range of diseases characterized by impairment of neuronal function during brain development. They have a strong genetic component; though they can result from a single mutation, they are more commonly multigenic (20). Ideally suited to modeling complex genetic diseases, iPSC-based models of neurodevelopmental disorders can recapitulate the early steps of neuronal differentiation in genetic

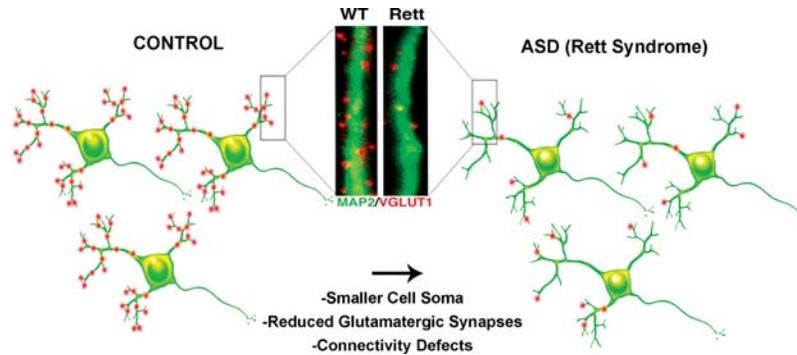


Figure 2. Example of neuronal deficits detected in ASD (RTT) patients. Rett neurons have smaller cell soma size; fewer glutamatergic synapses and altered connectivity when compared with neurons from non-affected (control) individuals. The panel in the middle depicts representative images from dendrites (MAP2) from WT (control) and Rett patient neurons derived from iPSCs. The red puncta (VGLUT1) on the green dendrites represent glutamatergic connections.

backgrounds known to result in neurodevelopmental disorders, permitting the study of the cellular and molecular causes of these disorders.

Autism spectrum disorders (ASDs) are complex neurodevelopmental diseases, highly heritable and characterized by deficits in impaired social interaction and repetitive behavior. The prevalence of ASD in the USA is currently estimated to be ~1:110 (21,22). The above-mentioned heritability, the suggested increase in prevalence and the current lack of early biological markers, relevant mouse models and effective treatments make ASD an attractive disease for future modeling with iPSC.

Rett syndrome (RTT) is a rare monogenetic disorder included in the ASDs and is caused by mutations on the methyl CpG-binding protein (*MeCP2*) gene. Two groups, including our own, recently showed relevant cellular phenotypes using iPSC-derived neurons from patients with RTT (23,24). Consistent with RTT animal models and RTT postmortem human brain tissue (25), both groups detected a decrease in cell soma size of RTT neurons compared with non-affected controls. Moreover, we also described a decrease in glutamatergic connections confirmed by electrophysiology, suggesting a communication problem in RTT neuronal networks (Fig. 2). Treatment with insulin growth factor 1, a growth factor known to ameliorate the phenotype of RTT mice, improved the RTT iPSC-neuronal phenotypes, providing evidence that synaptic defects can be rescued in neurons derived from RTT patients (26,27). In another study, neural progenitors derived from RTT iPSCs were used to analyze mobile element regulation via *MeCP2* loss of function, suggesting a new potential molecular mechanism of RTT (28). More research is necessary to determine whether iPSCs from patients with other forms of ASD share common cellular phenotypes with those from RTT patients and if those *in vitro* phenotypes are robust enough to be translated into clinically relevant drug screenings.

Fragile X syndrome (FXS) is an X-linked condition with variable expressivity; it encompasses a number of physical, intellectual, emotional and behavioral phenotypes that vary in severity. It is caused by expanded triplet repeats in the fragile X mental retardation (*FMR1*) gene, resulting in silencing of expression of *FMR1*, a gene believed to be involved in synapse formation. It is thought that, during neuronal

differentiation, full expansion of the triplet repeats results in hypermethylation of *FMR1* and chromatin modifications such as histone H3 deacetylation, histone H3K9 methylation and histone H3K4 demethylation (29,30). In human ESCs derived from FXS blastocysts identified via preimplantation genetic diagnosis, *FMR1* gene silencing occurs only upon ESC differentiation (31). Inconsistent with this finding, when skin and lung fibroblasts from three patients with FXS were used to create iPSCs (32), the reprogramming process failed to reverse the methylation of *FMR1*. Unlike both wild-type and FXS ESCs, in which the *FMR1* gene is active until differentiation, FXS iPSCs already have an inactivated *FMR1* locus. Consequently, the authors concluded that FXS iPSCs are not ideally suited to model the effects of *FMR1* silencing during neuronal differentiation, though they did not discuss whether neuronal phenotypes nonetheless occurred in FXS iPSC-derived neurons.

Schizophrenia (SCZD) is a debilitating psychiatric disorder that occurs in ~1% of the global population and is characterized by positive (hallucinations and delusions), negative (loss of affect) and cognitive symptoms. Though the overt clinical symptoms of SCZD generally appear during late adolescence or early adulthood, it is increasingly accepted that disturbed cognitive functions can occur well prior to disease onset (33). The first report of SCZD iPSCs were from patients with mutations in *DISC1*, an extremely rare monogenic form of SCZD. These iPSCs were generated using an integration-free method but have not yet differentiated into neurons (34). We recently reported generation of SCZD iPSC neurons from four patients with complex genetic cases of SCZD. We observed that SCZD iPSC neurons had reduced neuronal connectivity, reduced neurite outgrowths from soma, reduced PSD95 dendritic protein levels and altered gene expression profiles relative to controls; defects in neuronal connectivity and gene expression were ameliorated following antipsychotic treatment (35).

A key criterion in successful iPSC-based modeling of neurodevelopmental disorders is a high degree of heritability; a number of disorders, including bipolar disorder, Tourette's syndrome, attention deficit/hyperactivity disorder, obsessive-compulsive disorder, depression and addiction, meet this benchmark and should be good candidate disorders to be studied by this method. Of these, the disorders for which a specific subtype of neuron has already been

implicated, be it by histological or pharmacological methods, are perhaps more ideally suited. For example, postmortem human studies have already identified a decrease in GABAergic interneurons in the basal ganglia in Tourette's syndrome (36), serotonergic neurotransmitter activity is linked to depression (37) and dopaminergic neurons are linked to addiction (38). iPSC models may represent an exciting opportunity to examine the mechanism of disease specifically in these cells. Patient-derived iPSCs are an exciting new tool with which to test the pathogenic hypotheses of neurodevelopmental disorders and to begin linking mutations with specific biological pathways and developmental defects at the neuronal level.

Challenges in modeling neurological disease

iPSC technology has a clear potential for identifying the molecular mechanisms of an array of neurological diseases that currently have no cure or effective therapy. Nevertheless, there are a number of pressing issues that need to be addressed before iPSC technology can be extensively used for clinically relevant modeling of neurological diseases. Among these issues are variability in iPSC generation methods, variability between individuals, epigenetic/genetic instability and the ability to obtain disease-relevant subtypes of neurons.

Recently, researchers have begun to assess (and quantify) the variability that is present in iPSC lines. Increased levels of aneuploidy (39), defects in X-chromosome inactivation and genomic imprinting (40,41), aberrant epigenetic reprogramming (42), presence of point mutations and copy number variation differences (43,44) have all been detected in various iPSC lines. It is unclear that which of these differences might be relevant in iPSC disease modeling, as both the expected somatic variability and the level of genetic mosaicism observed within the lifetime of normal individuals remains unknown. Without this knowledge, we cannot yet fully judge the implications of the variability seen between iPSC cultures.

Genomic/epigenomic variability can influence the neuronal differentiation potential of iPSCs (45,46). This variability has been attributed to the use of randomly integrating viral vectors to introduce the reprogramming factors. However, it remains unclear whether novel non-integrating methods will decrease this variability or if the variability instead reflects inherent differences between iPSC lines (47–50). Nonetheless, many published reports have overcome this variance and detected significant phenotypic differences between neuronal cultures from patients with neurological diseases and unaffected controls. Gain- and loss-of-function studies, when possible, can verify that the phenotype observed is specific to the mutated gene and not due to acquired genetic/epigenetic variability.

Current protocols for differentiating iPSC into specific subtypes of neurons are under development. As researchers strive to identify ideal combinations and concentrations of growth factors critical to human neural development, clues can be found in mouse neural embryology studies, though adaptation is required as the developmental timing differs substantially between the two species. Understanding the molecular players involved in human neural differentiation will facilitate the development of methods and tools to enrich and monitor the generation of specific subtypes of neurons that would be more

relevant in modeling different neurological diseases. Particularly, promoter bashing techniques could be used to identify different subtypes of neurons for live imaging (51–53), and fluorescent-activated cell sorting using cell surface neuronal markers could be used to purify homogeneous populations (54,55). To date, greater progress has been made in generating enriched populations of ventral midbrain dopaminergic neurons that are relevant for PD (56,57) and spinal motor neurons that are important players during ALS pathology (13). Some progress has been made in regionalizing human ESCs into forebrain cholinergic neurons, often affected in AD, but iPSCs have not yet been subjected to these protocols (10,58).

Recapitulation of human corticogenesis *in vitro* has also been a challenge (59). Modeling for diseases where organization of cortical layers is proposed to be altered, such as Autism (60) and Schizophrenia (61), would benefit from protocols that accurately generate enriched populations of cortical neurons. Compartmentalization and stratification of neurons using chamber devices associated with live imaging would be useful to start teasing out the dynamic behavior and molecular anatomy of those neurons in a more refined way (62,63).

Recently, several groups have reported the direct conversion of somatic cells to post-mitotic neurons, skipping an iPSC intermediate (64–67). While promising, this technology could be limited by the subtypes of neurons generated, decreased efficiency and the finite proliferative capabilities of most somatic cell sources. Primary cells typically senesce after consecutive passaging, whereas iPSCs have nearly limitless replicative abilities.

CONCLUDING REMARKS

Modeling neurodegenerative and neurodevelopmental diseases using iPSCs has the potential to provide a valuable impact on modern medicine. These are the early days of iPSC technology. Optimal tools are still being developed along with mechanistic studies and continual validation. In conjunction with other techniques, such as mouse modeling, magnetic resonance imaging, whole-genome sequencing and longitudinal clinical data analysis, iPSC technology could play a genuine role in understanding neurological diseases.

ACKNOWLEDGEMENTS

The authors would like to thank J. Simon for illustrations and M.L. Gage for editorial comments.

Conflict of Interest statement. None declared.

FUNDING

The authors are funded by the California Institute of Regenerative Medicine (CIRM) (RC1-00115-1 and RL1-00649-1). F.H.G. is supported by The Lookout Fund, the Mathers Foundation and the Helmsley Foundation; M.C.M. is supported by Christopher and Dana Reeve Foundation (CDRF); K.J.B. is supported by a training grant from the CIRM.

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