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Modeling neurological diseases using patient-derived induced pluripotent stem cells

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Abstract

Reprogramming of somatic cells to an embryonic-like state has dramatically changed the landscape of stem cell research. Although still in its formative stages, the field of induced pluripotent stem cells (iPSCs) has the potential to advance the study of neurodegenerative and neurodevelopmental disorders at the molecular and cellular levels. The iPSC technology could be employed to establish *in vitro* experimental model systems for the identification of molecular lesions and to aid in the discovery of therapeutic targets and effective compounds. The derivation of patient-specific iPSCs has also opened up the possibility of generating disease-relevant cells for toxicity screening and for cellular therapy. In this article, we review the recent progress in the use of disease-specific iPSCs for *in vitro* and *in vivo* modeling of neurological diseases.

Keywords

disease modeling; human induced pluripotent stem cell; neurobiology; neurodegenerative; neurodevelopmental

In 2006, a landmark study by Takahashi and Yamanaka described the generation of a new category of stem cells known as induced pluripotent stem cells (iPSCs) [1]. The iPSCs were originally derived by retrovirally introducing four transcription factors: octamer-binding transcription factor 3/4 (*Oct3/4*), SRY-related high-mobility group box protein-2 (*Sox2*), *c-MYC* and Kruppel-like factor-4 (*Klf4*) into mouse embryonic and adult fibroblasts, which resulted in the reprogramming of these somatic cells into cells exhibiting a pluripotent phenotype. This scientific breakthrough was replicated using a variety of human somatic cells types, such as dermal fibroblasts, keratinocytes and lymphocytes [2–10]. Early characterization studies ascertained that despite the different origin of parental cells, iPSCs share fundamental properties with human embryonic stem cells (hESCs) including comparable morphology, self-renewal and proliferative capacity, telomerase activity, expression of stem cell genes and, most importantly, developmental potential [4,5]. Similar to hESCs, fully reprogrammed human iPSCs (hiPSCs) are capable of differentiating into any of the three primary germ layers and could provide an unlimited source of differentiated cell types [11]. Although still in its formative stages, the generation of pluri potent stem cells from somatic cells has created much excitement within the scientific community. hiPSCs have already opened up a myriad of experimental possibilities for both research and

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potential clinical applications, including cell-based therapies utilizing custom-designed cells derived from individual patients and the generation of well-defined *in vitro* systems from patient-specific cells that could be used to identify molecular lesions, elucidate the pathogenesis of diseases and discover clinically relevant therapeutic targets. This article will provide a summary of the use of iPSC technology to advance the study of neurological disorders, focusing on employing hiPSCs for *in vitro* modeling of neurodegenerative and neurodevelopmental diseases.

hiPSC generation

Derivation of hiPSCs

The first generation of hiPSCs was engineered using retroviruses and lentiviruses to introduce a combination of reprogramming transcription factors into somatic cells [4,5,8]. Both retroviral and lentiviral vectors allow for transgene insertion into the host cell genome and ectopic expression of the transcription factors required for the reactivation of endogenous genes that are necessary for pluripotency. While these vectors are still the most effective methodology to induce reprogramming, there are several disadvantages of using retroviral and lentiviral gene delivery systems for hiPSC generation [12,13]. The presence of multiple proviral integration sites combined with the possibility of incomplete silencing or reactivation of the transgenes increases the likelihood for tumorigenesis, particularly when *c-MYC*, a proto-oncogene, is utilized in the reprogramming cocktail [14–16]. Furthermore, there is also an inherent risk of malignant transformation by insertional mutagenesis when using retroviral or lentiviral vectors for gene transfer [17].

The technology for hiPSC generation has been moving at an accelerated pace, with the goal of developing an optimal method for hiPSC derivation. Reprogramming strategies have emerged that emphasize the need for safety and the desire to produce hiPSCs with minimal disturbance to the host cell genome. In an attempt to reduce the oncogenic potential of the conventional Yamanaka reprogramming factors (*Oct3/4, Sox2, c-MYC* and *Klf4*), hiPSCs have been derived using only three of the four factors, excluding the *c-MYC* transgene [18,19]. It has been shown that high-quality, fully reprogrammed iPSCs can be generated without ectopic *c-MYC*, albeit at a substantially lower efficiency (<0.001%) [18]. hiPSCs have also been generated by replacing *Klf4* and *c-MYC* with *Lin28* and *NANOG* transgenes in the four-factor cocktail [4]. In addition, hiPSCs have also been derived by overexpressing *Sox2* and *Oct4* in the presence of valproic acid, a histone deacetylase inhibitor, at a similar efficiency to the three-factor cocktail [20]. The induction of pluripotent stem cells in the absence of *c-MYC* has provided fundamental information regarding the biology of reprogramming, and moved the field one-step closer to the generation of safer hiPSCs.

To circumvent issues related to the presence of multiple proviruses throughout the host cell genome, nonintegrating viral vectors (e.g., adeno-viruses and Sendai virus) and physical methods of gene transfer (e.g., electroporation of plasmids that remain episomal) have been explored as means to derive hiPSCs [7,21–26]. Although transient expression of reprogramming transcription factors by nonintegrating methods can successfully produce *bona fide* hiPSCs, the efficiency in general is extremely low, with 0.0002% for adenoviral induction [25] and between 0.0003 and 0.0006% for episomal vector-based reprogramming [22]. As a result, these methods are very labor intensive when using fibroblasts as the target cell population [27]. Enhanced efficiency of episomal-based reprogramming has been reported with the use of human fetal neural progenitor cells as the starting material [28]. The neural stem cells have endogenous expression of *Sox2* and *c-MYC*, thus requiring only the addition of *Oct4* and *NANOG* for reprogramming to occur [28].

A single nonviral vector encoding all four Yamanaka factors has been used to derive hiPSCs in combination with *piggyBac* transposons that can eliminate the plasmid from the cell genome once reprogramming has occurred [29,30]. While this system is an improvement from the use of lentiviral and retroviral vectors as it allows for complete removal of the reprogramming factors, it still requires genomic integration for induction to occur. Another successful yet inefficient strategy is the direct delivery of membrane-permeable tagged recombinant proteins and synthetic mRNAs of reprogramming factors [31,32]. More recently, a transgene-free chemical method that relies on the administration of small molecules for stem cell induction instead of the introduction of transgenes has been developed [33,34]. This obviates the need for genetically manipulating the genome and has been shown to markedly increase the efficiency of reprogramming (>200-fold) [33]. While this method appears to be a major advancement in hiPSC generation, it has yet to be determined whether the cells experience any genetic abnormalities as a result of the chemical exposure. Despite the rapid progress, it is clear that further refinement of hiPSC generation technology is needed to increase the reprogramming efficiency and to ensure the generation of safe, fully reprogrammed stem cells.

Characterization of hiPSCs

Validating the complete reprogramming and confirming the developmental functionality of hiPSCs is an extensive, arduous and unavoidable task [35]. The selection and identification of a fully reprogrammed hiPSC colony is critical and entails rigorous characterization of the stem cell clones due to the existence of a high percentage of incompletely reprogrammed cells [11,35]. As partially reprogrammed colonies may have impaired differentiation or induced tumor formation, it is essential to exclude these cells from further experiments [1,14,36]. Colonies are initially selected based on having a similar morphology to hESCs. Next, the standard assays of alkaline phosphatase staining, detection of pluripotency markers, assessing DNA methylation status of promoters of pluripotent genes, confirming retro viral silencing and cytogenetic analysis are completed [5,35]. One hallmark characteristic of hESCs and hiPSCs is their pluripotency, or developmental potential to differentiate into all cell types in the human body [37,38]. To evaluate the developmental capability of hiPSCs, the cells are differentiated *in vitro* into various mature cell types representing the three germ layers, typically using an embryoid body intermediate. However, *in vitro* differentiation is not considered as sufficient to conclude that a hiPSC line is truly pluripotent. When injected into immunocompromised mice, the hiPSC must give rise to tumors that contain cell types originating from the mesoderm, ectoderm and endoderm; this is an *in vivo* assay known as teratoma formation [39]. The standards for demonstrating pluripotency for hiPSCs differ from the characterization of mouse iPSCs where the generation of germline-competent chimeric mice is often used, as well as the most stringent test for pluripotency using tetraploid complementation to obtain viable mice [40,41]. Both of these analyses are unavailable for hiPSC research due to ethical concerns.

Differentiating hiPSCs into cells of the nervous system

A distinct advantage of using patient-derived hiPSCs for *in vitro* disease modeling is the ability to produce differentiated cell types that are directly relevant to the disease pathology. For researchers interested in using hiPSCs to model neurological diseases, protocols for differentiating hESCs into major cell types of the nervous system, including neurons, astrocytes, oligodendrocytes and Schwann cells, have been developed and fortunately can often be adapted for hiPSC differentiation [42–46]. The majority of hESC differentiation protocols are time consuming, laborious and complex, requiring frequent substrate and medium changes, supplementation with expensive growth factors/morphogens and sometimes the incorporation of stromal cell lines for neuronal induction [44,45,47,48]. For many neurological diseases, one specific neural subtype is more susceptible to disease

insults, such as motor neurons in amyotrophic lateral sclerosis (ALS), dopaminergic neurons in Parkinson's disease (PD) and medium spiny neurons in Huntington's disease (HD) [49]. Thus, much effort has been devoted to generating specific types of neurons using chemically defined conditions that require precise temporal exposure, typically of long duration (ranging from weeks to months). For example, dopaminergic neuron differentiation from hESCs can range from 21 days to more than 2 months in culture [50,51]; functional motor neuron differentiation requires 8–10 weeks [52] and medium spiny neuron differentiation takes over 60 days [53]. The ability to generate specific types of neurons from patient-derived hiPSCs holds much promise for modeling human neurological diseases and to understand the molecular and cellular mechanisms underlying neuropathology. However, technical hurdles still plague hESC protocols, such as generating a homogenous cell population or sufficient numbers of a neuronal subtype. Much work still needs to be done to improve the efficiency and efficacy of differentiating hiPSCs into various neural sub-types. Assessing individual hiPSC lines through direct comparison with hESC differentiation efficiency is key to determining whether hiPSCs will respond in an equivalent manner. It is known that hESC lines differ in their propensity to form particular germ layers [54]. As hiPSCs closely resemble hESCs, hiPSCs are likely to exhibit differences in their tendency to differentiate into neural progenitors and their various mature progeny. It has been shown that hiPSCs differentiate more efficiently into cells that resemble the somatic cells from which the hiPSCs were derived [55,56]. The significant variability in the differentiation capabilities of individual hiPSC clones can either facilitate or hinder the development of an *in vitro* model system. Thus, the identification of markers that would predict the ability of a hiPSC line to form specific cell lineages would be extremely useful [57]. The intrinsic developmental heterogeneity of hiPSCs has been investigated in a study in which the neural developmental potential of 12 hiPSC lines derived by traditional viral-based methods as well as nonintegrating vectors were compared with five hESC lines [42]. As expected, hiPSC lines are able to respond to patterning signals, generate functional neurons and follow a similar developmental time course as the hESC lines. However, the neuronal differentiation efficiency of hiPSCs was lower than that of hESCs and variable between the cell lines. Surprisingly, differences were not observed between transgene-derived hiPSCs and transgene-free hiPSCs. These data provide evidence favoring the use of hiPSCs for the study of neurological disorders, with the caveat that continued optimization of differentiation protocols is imperative to enhancing the efficiency of neuronal generation.

In vitro disease modeling of neurological disorders

Utilizing hiPSCs for disease modeling

The derivation of hiPSCs from diseased patient cells has the potential to be a powerful tool for studying neurological diseases at the cellular level, whether they are defined genetic disorders (e.g., HD) or complex diseases (e.g., ALS and schizophrenia). The routine acquisition and banking of primary cells from patients with neurological diseases could therefore provide an invaluable resource to derive an unlimited supply of isogenic pluripotent stem cells. Tissues that are easily accessible, such as skin, hair or blood, are prime candidates as starting material for hiPSC derivation. Once hiPSC lines have been established, they can be maintained indefinitely and used to generate a diverse repertoire of differentiated cell types, including neural progenitors, neurons and glia **(Figure 1)**.

Disease-specific hiPSCs have been derived from patients with a variety of neurological diseases and used to produce a number of neuronal subtypes [58–63]. The neurons or glia generated from hiPSCs could be used as substrates for high-throughput screening in order to identify therapeutic targets and drug sensitivities, to validate drug safety or to aid in biomarker development. Mechanistic studies using hiPSCs derived from patients with complex disorders, such as PD and Alzheimer's disease, may facilitate the identification of

genes that play pivotal roles in disease pathogenesis or reveal novel roles for genes linked to the disorder. For diseases with known genetic mutations such as HD, the development of an *in vitro* model system using both normal and diseased hiPSCs would allow for systematic evaluation of the functional consequences of the mutation in specific cell types, such as medium spiny neurons. Furthermore, as HD is a trinucleotide-repeat disorder having clinical phenotypes that differ with the length of the expanded repeat, polyglutamine repeat-length-dependent pheno-types may be detected when comparing cells from patients with varying CAG repeats. Therefore, the creation of patient-specific pluripotent cells enables the generation of a diverse set of experimental platforms that can be applied to study human diseases.

Successful hiPSC disease modeling

Although human somatic cell reprogramming was first reported in 2007 [4,5], to date, there are few studies that have effectively implemented the use of hiPSCs for modeling neurological diseases **(Table 1)**. Initial experiments established the feasibility of deriving hiPSCs from skin fibroblasts acquired from patients with ALS, HD, PD, Down syndrome and schizophrenia [27,58,59,61]. Specific neuronal subtypes were generated, such as motor neurons from ALS hiPSCs and dopaminergic neurons using PD hiPSCs; however, neuronal dysfunction or other abnormal neuronal phenotypes have not been reported [59,61]. Notably, increased caspase activity upon growth factor withdrawal was observed in neural progenitors derived from HD hiPSCs, yet abnormalities were not identified in HD hiPSC-derived striatal neurons [64]. The lack of detectable phenotypes at the mature neuron stage for the PD, ALS and HD hiPSCs emphasizes the difficulty in studying late-onset neurodegenerative disorders in a cell culture model system where long-term culture maintenance is not feasible.

On the other hand, encouraging results have emerged from the generation of hiPSCs from patients with neurodevelopmental disorders, including spinal muscular atrophy (SMA), familial dysautonomia (FD) and Rett syndrome (RTT) [60,65,66]. SMA is an inherited neuro-muscular disorder attributed to mutations in the survival motor neuron (*SMN1*) gene and reduced levels of the corresponding protein, SMN. Shortage of this protein results in the degeneration of lower motor neurons in the spinal cord and brain stem. The potential to use patient-specific pluripotent stem cells for drug screening was initially illustrated in a study that used SMA hiPSCs to validate SMN protein-inducing compounds [60]. In addition to using SMA hiPSCs to confirm that valproic acid and tobramycin could increase SMN protein levels, the diseased stem cells were used to generate motor neurons, the cell type that is primarily affected by the loss of SMN proteins. Compared with wild-type hiPSC-derived motor neurons, SMA motor neurons are reduced in size and decreased in number under prolonged culture conditions. These findings suggest that the SMA-derived motor neurons had abnormal survival or production. This study was the first to demonstrate a pathologic phenotype using hiPSCs and to provide evidence to support the feasibility of applying hiPSCs for disease modeling.

The successful use of hiPSCs for disease modeling has also been demonstrated using pluripotent cells derived from patients with FD, a disease that affects the peripheral nervous system and is characterized by the loss of sensory and autonomic neurons [65]. While the pathogenesis of this rare autosomal recessive disease is unclear, it is known that causative mutations in the I-κ-B-kinase complex-associated gene (*IKBKAP)* results in aberrant mRNA splicing and decreases IKAP protein. Disease-related phenotypes were detected upon differentiation of FD hiPSCs into neural crest precursors. Striking differences were noted in the ability of FD hiPSC neural crest precursors to migrate and to differentiate into neurons. In addition to functional abnormalities, the FD hiPSC-derived neural crest precursors also exhibit reduced expression of normal *IKBKAP* transcripts. The FD neural precursors have

been used to screen a series of drugs with known effects on *IKBKAP* splicing. Remarkably, one of the compounds – kinetin – was found to reduce the mutant transcript and increase normal *IKBKAP* levels. This drug was also able to incompletely restore one of the abnormal pheno types by increasing FD hiPSC neural crest neuro genesis. The discovery and partial reversal of disease-related defects in FD hiPSC progeny demonstrates the utility of the cell culture model to validate candidate compounds.

A more elaborate study utilizing disease-specific hiPSCs boight about the development of an *in vitro* model of RTT, a neurodevelopmental, X-linked disorder typified by autistic behavior and apraxia [66]. Patient fibroblasts carrying various causative mutations in the methyl CpG binding protein 2 (*MeCP2*) gene were used to derive hiPSCs. Marked morphological and functional differences were detected in neurons derived from RTT hiPSCs. The diseased glutamatergic neurons have fewer dendritic spines, reduced numbers of synapses and smaller somas compared with neurons derived from unaffected control hiPSCs. Electrophysiological abnormalities and calcium signaling defects were also observed. The RTT-affected neurons were used to evaluate candidate compounds, and two drugs were found to rescue the reduced synapse phenotype exhibited by RTT hiPSC-derived neurons. In addition to pharmacologic treatments, investigators used lentiviral vectors encoding shRNA against *MeCP2* to confirm the role of *MeCP2* in the observed phenotype. The remarkable neuronal alterations detected with this model system strengthen the notion that disease-specific phenotypes can be identified using hiPSCs and warrant their use for future drug screening.

Concerns & obstacles to using hiPSCs for neurological disease modeling

With advances in pluripotent stem cell generation, the derivation of patient-specific hiPSCs will become a standard procedure that can be accomplished in laboratories with experience in molecular and tissue culture techniques. However, effectively utilizing the diseased cells for modeling neurological diseases remains challenging. To facilitate the accurate assessment of a disease phenotype *in vitro*, the generation and utilization of proper hiPSC control lines is also critical. The paucity of hiPSC studies clearly demonstrating a robust disease-related phenotype provides evidence that this is a complex and intricate undertaking, particularly for neurodegenerative diseases. Adult-onset disorders that clinically manifest after several decades may be difficult to mimic *in vitro* within a practical experimental time period. Attempting to recapitulate the kinetics of a late-onset disease is likely to be a complicated procedure demanding supplementary stressors to accelerate the progression. For disorders that have non-cell-autonomous contributions such as ALS [52,67,68], there is a requirement to generate extra cell types that are essential to mediating the toxicity, adding another layer of complexity to the experimental system and requiring extensive optimization of differentiation protocols and culture conditions. Even the appropriate cell types can be produced; the cell culture model may still be deficient in the micro-environmental factors that modulate the disease process, which may result in either a subtle or absent phenotype.

Additional concern for using hiPSCs for disease modeling is the disease specificity; for instance, whether extensive passaging or prolonged culture of HD hiPSCs and their derivatives would result in CAG repeat-length instability, and what influence the polyglutamine expansion would have on the model system. Triplet-repeat instability has recently been reported for hiPSCs derived from patients with Friedreich's ataxia with inter-generational contractions and expansions observed within the *FXN* gene [69]. This study reinforces the concept that when developing a model system, the intrinsic properties of the diseased cells may necessitate the examination of extra parameters to ensure the quality of the hiPSCs that are generated. A similar finding of repeat-length instability is possible with HD-derived hiPSCs; consequently, periodic genotyping may be mandatory to monitor for this occurrence.

Another variable to consider when generating hiPSCs for neurological disorders is that the disease mutation may create barriers to efficiently deriving stable cell lines. It has been documented that dermal fibroblasts from patients with Faconi anemia, a chromosomal instability disorder, are more resistant to reprogramming [70]. Genetically correcting the mutated somatic cells prior to the induction of pluripotency was critical for establishing disease-specific hiPSCs. This phenomenon has not yet been reported for neurological diseases; however, it should be considered if faced with consistent reprogramming difficulties.

Using hiPSCs in conjunction with animal models to study neurological diseases

Rodent models have been instrumental in providing insights into human neurodegenerative and neurodevelopmental diseases. Although essential knowledge has been gained from the use of animal models, there are limitations in their ability to fully replicate all aspects of human physio logy and neuropathology. Currently, hiPSCs have largely been used as a complementary *in vitro* approach to model human diseases. However, they may also be employed in combination with rodent models to enhance animal systems and perhaps yield more translational information. Experiments have been performed to address whether differentiated cell types generated from patient-specific hiPSCs could be transplanted into rodent models for therapeutic purposes [71]. These studies build on previous experiments that assessed the ability of hESCs to integrate and differentiate in the developing mouse brain [72]. Researchers using hiPSCs derived from PD patients determined that transplanted differentiated cells were able to survive, engraft and differentiate into dopaminergic neurons within the rat striatum for at least 12 weeks [71]. When PD hiPSC-derived cells were transplanted into the striatum of rats treated with 6-hydroxydopamine, functional effects were noted as the rats exhibited less amphetamine-induced rotations compared with lesioned rats without transplantation. Long-term survival of the transplanted cells was also detected as the cells are present at 16 weeks postinjection. Evidence of PD pathology, such as inclusion body formation, was not observed in the transplanted neurons of either untreated or lesioned animals. Similar studies assessing the engraftment ability of hiPSC-derived cells have been conducted with normal hiPSCs and the mouse eye as the target organ [73]. Photoreceptor cells derived from hiPSCs and transplanted into the sub retinal space were able to survive and integrate into the retina and express markers of photoreceptors. As the hiPSCs were transplanted into wild-type mice without any ocular lesions, the ability of the cells to restore light responses was not evaluated. These reports provide plausible evidence that the transplantation of hiPSC-derived cells into rodent models can be developed into a unique bioassay to evaluate the *in vivo* functional capabilities of hiPSCs. If disease phenotypes can be recapitulated, this approach can be used to generate preclinical models to test the efficacy of potential therapies.

Conclusion

There is much excitement surrounding the derivation of hiPSCs for basic and clinical research. The generation of disease-specific hiPSCs will undoubtedly be an important tool to investigate a multitude of human diseases at the cellular level; however, it is imperative that hESCs continue to be utilized and studied in parallel. It has been established that hiPSCs and hESCs have transcriptional and epigenetic differences, and additional analyses are required to determine how truly interchangeable the two cell types are [74–78]. At the present moment, hESCs are the gold standard with which hiPSCs are compared. Until the biology of hiPSCs is more thoroughly understood, hESC experimentation should be sustained. The few reported studies using patient-specific hiPSCs for disease modeling have confirmed that reprogrammed cells are a unique and versatile resource that is poised to make an exceptional

contribution to the study of neurobiology and neurological disease mechanisms. The reprogramming of somatic cells to an embryonic-like state has dramatically changed the landscape of stem cell research, and this outstanding achievement has paved the way for a new era in biomedical research.

Future perspective

Currently, the generation of patient-specific cells for disease modeling has been a major focus for the development of hiPSC technology. The field of regenerative medicine is embracing the possibility that hiPSCs may some day supplant embryonic stem cells for cell-based therapy and personalized medicine. While allogeneic transplantation has been used successfully in treating human patients (e.g., bone marrow transplantation), the production of patient-specific hiPSCs could provide an attractive alternative due to the possibility of creating autologous grafts with reduced immunogenicity [79]. With the rapid technical advancement and immense research effort directed towards the generation of safe, functionally competent cells that are free of genetic aberrations and devoid of tumorigenicity, we are expecting that some day patients will benefit from hiPSC-based cell therapies.

Another blossoming area of research is the generation of disease-corrected patient-specific hiPSCs with known genetic defects [80]. The insertion of a therapeutic gene into the genome of stem cells by homologous recombination or by viral vectors has been accomplished in the field of gene therapy. Advancements have been made to adopt these strategies to efficiently target specific genes in hiPSCs by implementing a zinc-finger nuclease-mediated process to initiate homologous recombination [81]. As proof-of-principle, a recent study used a lentiviral vector for gene correction of diseased hiPSCs derived from Fanconi anemia patients [70,82]. This study combined two powerful technologies to produce genetically modified cells from diseased patients, resulting in amelioration of the disease phenotype. With continued research in combining gene therapy and hiPSC technology, the generation of clinical grade, disease-corrected hiPSCs for therapeutic cell replacement studies may become a reality.

Finally, the reprogramming of somatic cells into cells with an embryonic-like state has stimulated research into the direct induction of somatic cells into specific differentiated cell types, bypassing the iPSC stage. Recent studies in mice have demonstrated that direct reprogramming is a viable approach for deriving mature, specialized cells [83–85]. Pancreatic exocrine cells have been directed *in vivo* to convert into pancreatic β-cells by virally expressing a combination of three transcription factors that are critical for pancreatic development [83]. Interestingly, three developmental transcription factors were also identified that initiated the conversion of mouse cardiac and dermal fibroblasts into functional cardio myocytes [84]. Similarly, only three factors – *Ascl1*, *Brn2* and *Myt1l* – were deemed sufficient for efficient *in vitro* conversion of mouse fibroblasts into functional neurons that have the ability to form synapses and generate action potentials [85]. The transformation of one differentiated cell type into another specialized cell type by direct reprogramming has yet to be reported using human somatic cells. However, given the success of hiPSC technology, it appears to be an achievable goal. The conversion of human somatic cells into neurons would enable one to bypass the pluripotent stem cell stage and the difficulties associated with generating and maintaining hiPSCs, such as the extensive and lengthy characterization assays and the possibility of teratoma formation when using hiPSC-derived cells for cell-based therapy. Disease-specific neurons produced by this alternative method could be used in a similar fashion to hiPSC-derived neurons for pathogenesis and therapeutic studies and as a means to validate any phenotype observed using hiPSC-generated neurons. The developmental plasticity uncovered by this novel approach could

also be used to broaden our understanding of the regulators of neuronal development. One limitation of direct conversion from one differentiated cell type to another differentiated cell type is the lack of a renewable cellular source. In light of the rapid progress in cell reprogramming, we expect to see reprogramming of somatic cells into neural stem cells, which maintains the capacity for long-term expansion due to their ability for self-renewal.

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Executive summary

Human induced pluripotent stem cell generation

- Somatic cell types, including fibroblasts, keratinocytes and lymphocytes, have been used as starting material for human induced pluripotent stem cell (hiPSC) derivation.
- hiPSCs were first engineered using retroviruses to deliver key transcription factors that are critical for reprogramming into somatic cells.
- High-quality, fully reprogrammed cells have been produced using nonintegrating methods, such as adenoviruses, episomal-based vectors and small molecules.
- The selection, validation and characterization of fully reprogrammed hiPSCs is a labor-intensive task involving numerous assays.

Differentiating human induced pluripotent stem cells into cells of the nervous system

- hiPSCs have been differentiated into glia and various neuronal subtypes (motor, dopaminergic and striatal).
- hiPSCs can respond to patterning signals and follow a similar neuronal developmental time course as human embryonic stem cell lines.

In vitro disease modeling of neurological disorders

- Derivation of hiPSCs from diseased patient cells has the potential to be a powerful tool to study neurological disorders at the cellular level.
- Neurons generated from hiPSCs could be used for mechanistic studies aimed at elucidating the pathogenesis of neurological diseases or could be utilized in biomarker and drug development.
- Disease phenotypes have been difficult to detect at the mature neuron stage for late-onset neurodegenerative disorders, such as Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease.
- Successful use of hiPSCs for disease modeling has been demonstrated using pluripotent stem cells derived from patients with neurodevelopmental disorders, including spinal muscular atrophy, familial dysautonomia and Rett syndrome.
- Modeling neurological diseases using hiPSCs can be challenging when studying adult-onset neurodegenerative diseases or disorders with non-cell-autonomous contributions.

Using human induced pluripotent stem cells in conjunction with animal models to study neurological diseases

- Rodent models of neurological diseases may be enhanced by the incorporation of hiPSCs into the experimental system.
- The transplantation of hiPSC-derived cells into rodent models can be developed into a unique bioassay to evaluate the *in vivo* functional capabilities of hiPSCs and determine their therapeutic potential.

Conclusion

- hiPSC technology has the potential to advance the study of neurological diseases by facilitating the development of well-defined *in vitro* experimental systems for the identification of molecular lesions and to aid in the discovery of therapeutic targets.

■ The derivation of patient-specific hiPSCs has also opened up the possibility of generating custom-designed cells for regenerative medicine.

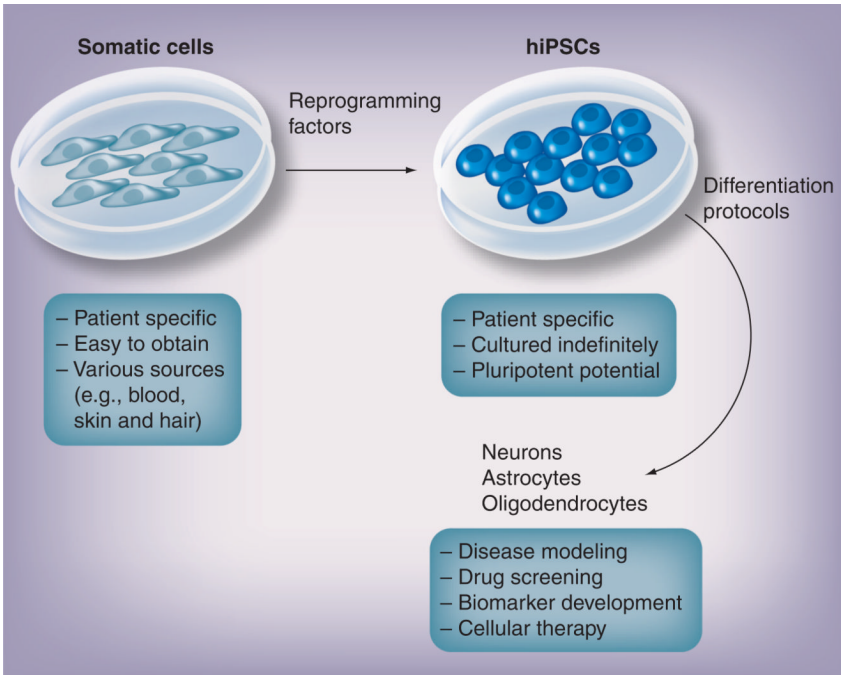

Figure 1. Workflow diagram for generating and using patient-specific human induced pluripotent stem cells

Somatic cells derived from diseased patients are directed to become pluripotent stem cells by the introduction of reprogramming factors. Once induced pluripotent stem cell lines are established, they can be maintained in culture or utilized in specific differentiation protocols. Mature progeny derived from hiPSCs, such as neurons, astrocytes or oligodendrocytes, can be employed for various experimental assays, including the development of *in vitro* models for studying neurological diseases. hiPSC: Human induced pluripotent stem cell.

Table 1

Induced pluripotent stem cells generated from patients with neurological diseases.

Disease	Genetic mutation	Differentiated cell type generated	Phenotype	Ref.
Amyotrophic lateral sclerosis	Superoxide dismutase gene	Motor neurons and astrocytes	None	[59]
Angelman syndrome	Maternal deletion of chromosome 15q11–q13	Neurons/astrocytes	Paternal repression of *UBE3A* expression in neurons	[62]
Down syndrome	Trisomy 21	Embryoid body formation	None	[58]
Familial dysautonomia	*IKBKAP* mutations	Neural crest precursors	Migration defects	[65]
		Neurons	Decreased neurogenesis	
Fragile × syndrome	CGG triplet repeat in *FMR1* gene	Phenotype in hiPSCs	Inactive *FMR1* locus	[63]
Friedreich's ataxia	GAATTC triplet repeat in *FXN* gene	Phenotype in hiPSCs	Repeat-length instability in culture	[69]
Huntington's disease	Expanded CAG repeat in *HTT* gene	Embryoid body formation	None	[58]
Huntington's disease	Expanded CAG repeat in *HTT* gene	Neural progenitors	Increased caspase activity upon growth factor withdrawal	[64]
		Striatal neurons	None	
Parkinson's disease	Unknown	Embryoid body formation	None	[58]
Parkinson's disease	Unknown	Dopaminergic neurons	None	[61]
Rett syndrome	MeCP2 mutations	Glutamatergic neurons	Fewer dendritic spines and number of synapses, smaller somas and reduced frequency of postsynaptic currents	[66]
Spinal muscular atrophy	*SMN1* gene mutation	Motor neurons and astrocytes	Reduced size and decreased number of motor neurons	[60]
Schizophrenia	*DISC1* gene mutation	Not reported	Not reported	[27]

hiPSC: Human induced pluripotent stem cell.