Cell Stem Cell
Perspective

Therapeutic Translation of iPSCs for Treating Neurological Disease

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Somatic cellular reprogramming is a fast-paced and evolving field that is changing the way scientists approach neurological diseases. For the first time in the history of neuroscience, it is feasible to study the behavior of live neurons from patients with neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, and neuropsychiatric diseases, such as autism and schizophrenia. In this Perspective, we will discuss reprogramming technology in the context of its potential use for modeling and treating neurological and psychiatric diseases and will highlight areas of caution and opportunities for improvement.

Introduction

Widespread use of reprogramming and programming technology is challenging our view of differentiated cells as irreversible entities. From the early works of Briggs and King (Briggs and King, 1952) and Gurdon (Gurdon et al., 1958) to the widespread advent of induced pluripotent stem cells (iPSCs) by Takahashi and Yamanaka (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yamanaka, 2012), we are now faced with the remarkable idea that all cells in our body maintain an intrinsic plasticity for differentiating into a variety of cell types with completely different functions. The impact of this technology has been most strongly felt in the neurosciences. While much work remains to be done to improve and refine the technology, attempts to apply these techniques to the clinic are already underway. One could argue that it is too early to consider translational research because much more basic understanding of its implications is required, but some of the applied approaches are pushing the field forward, resulting in the need for better systematic safety and reliability standards. Undoubtedly, much more work is needed to optimize iPSC technology. In this Perspective, we will discuss reprogramming technologies and their potential uses for modeling and treating neurological and psychiatric diseases, as well as highlighting areas of caution and opportunities for improvement.

Modeling Neurological and Psychiatric Diseases In Vitro with Pluripotent Stem Cells

Neurological and Psychiatric Diseases Currently Being Modeled with Patient-Derived iPSCs

Soon after human cells were first reprogrammed (Takahashi et al., 2007), a number of groups used the technology to model neurodevelopmental and neurodegenerative diseases. Neurogenetic disorders were modeled first (Dimos et al., 2008; Lee et al., 2009; Marchetto et al., 2010; Zhang et al., 2010), followed by a few examples of sporadic and complex disorders (e.g., schizophrenia [SCHZ] [Brennand et al., 2011; Paulsen et al., 2012; Pedrosa et al., 2011]), providing important insights into disease biology and potential therapeutic avenues (see Table 1 for references, description of diseases, and rescuing drugs). From these studies of neurodevelopmental/neuropsychiatric diseases, a general pattern has emerged regarding the inability of neurons to establish proper connections. Specifically, inadequate neuronal maturation, synaptic deficiency, and failed connectivity have been observed in many of the early-onset and neurodevelopmental diseases modeled so far (examples: familial dysautonomia [FD] [Lee et al., 2009], Rett syndrome [RTT] [Marchetto et al., 2010; Ricciardi et al., 2012], Huntington's disease [HD] [Chae et al., 2012], SCHZ [Brennand et al., 2011]). On the other hand, human iPSCs from patients with neurodegenerative disorders, while considered to be suitable for modeling neurodegenerative disorders, do not always exhibit the neuronal maturation and network defects that are observed in vivo. It is possible that this apparent identification of synaptic deficits may be in part because these are the measurements that have been focused on so far. In neurodegenerative diseases and proteopathies, neuronal toxicity due to increased sensitivity to oxidative damage and proteasome inhibition seems to be more prevalent than strictly synaptic deficits. Examples of these diseases include amyotrophic lateral sclerosis (ALS) (Mitne-Neto et al., 2011), Parkinson's disease (PD) (Nguyen et al., 2011), Alzheimer's disease (AD) (Israel et al., 2012), and Down syndrome, which mimics some aspects of AD (Shi et al., 2012). As the number of patients and types of neurological diseases being modeled increases, new patterns will emerge that could aid in the development of earlier diagnostic tools and facilitate effective drug design. Significant interest is growing among clinicians and the pharmaceutical industries as additional neurological conditions are proposed to be modeled using iPSCs. Attractive candidate diseases include, but are not restricted to, major depression, migraine, attention deficit hyperactivity disorder (ADHD), and idiopathic autism.

Major Challenges in Modeling Neurological and Psychiatric Disease and Tools for Addressing Them

When developing in vitro models, the main goal is to establish a meaningful parallel between the phenotypes observed in the dish and the disease pathology observed in vivo. An important set of challenges that currently surround this field involve the variability between clones and changes in clone genome and phenotype over passage and time. Targeted genome modification of human pluripotent cells using engineered constructs like zinc-finger nucleases (ZFNs) (Kim et al., 1996; Porteus 2010), transcription activator-like effector nucleases (TALENs)

(Christian et al., 2010; Bedell et al., 2012), and, more recently, the clustered regularly interspaced palindromic repeats/CRISPR-associated (CRISPR/Cas) system (Wiedenheft et al., 2012; Mali et al., 2013) present promising strategies to model monogenic and genetically defined disorders with reduced variability by generating isogenic control lines harboring defined genetic alterations (Soldner et al., 2011). These techniques are discussed in detail by Merkle and Eggan (2013) in this issue. However, these approaches are of limited use for modeling sporadic cases of diseases or complex neuropsychiatric disorders where there is no clear genetic etiology. It is conceivable that identifying protocols that generate lineage-specific cells will solve this problem by allowing investigators to monitor the differentiation process more specifically. Defining and consistently obtaining the disease-relevant neural cells at comparable levels of maturation should greatly reduce the phenotypical variability and highlight pertinent disease characteristics. Assessing neuronal network connectivity formation is important for understanding neuronal communication imbalance in disease but it can be a challenging task because as a general rule the right subtype of neurons and the specific maturation time are not represented in the dish at appropriate levels. To that end, promoter-bashing technology may aid in generating the desired populations of neurons that are directly involved in the disease being studied (for example, Hb9-positive cells for disease involving alpha motor neurons such as ALS [Dimos et al., 2008; Mitne-Neto et al., 2011] or TH-positive dopaminergic neurons for PD [Devine et al., 2011; Nguyen et al., 2011; Jiang et al., 2012; Peng et al., 2013]). Additionally, single-cell expression profiling should further clarify the levels of population heterogeneity within in vitro cultures, and advances in media culture platforms and automated cell processing should provide the desired accuracy and consistency that will be required.

For a number of neurological diseases, it remains unclear whether the phenotypes involved in the pathology are restricted to the neuronal population and to what extent the neighboring cells are also playing a major role. Improving the protocols for generation of cells present in the neuronal niche (i.e., astrocytes, oligodendrocytes, microglia, endothelial cells) could reveal important disease phenotypes and contribute to the development of alternative therapies. Refining the techniques to analyze neuronal phenotypes will also help to detect more subtle differences. Examples of techniques that have not been widely explored for neuronal characterization are light-activated channelrhodopsins, uncaged glutamate, transynaptic labeling using virus or dyes, multielectrode arrays, spine motility, high-resolution electron microscopy, axon protein transport dynamics, organelle activity and mobilization, and microfluidics devices for cellular compartmentalization. The field is becoming interdisciplinary, bringing together technological advancements from multiple areas including electrical and mechanical engineering with principles of neuroscience and stem cell biology. In the following sections, we briefly discuss two laboratory-on-a-chip technologies, microfluidics and microelectrode arrays (MEA), that have the potential to assist researchers in achieving these goals.

Finally, we posit that many of the challenges to in vitro disease modeling arise from the overall strategy employed. Many of the current disease modeling studies search for differences in gene expression generally or for basic functions that can be measured in vitro that have been hypothesized to be correlated causally in the disease. Often these studies are not hypothesis driven but rather depend on existing techniques and the availability of somatic cells from whatever patients are available to the researcher. However, researchers are increasingly working more closely with the clinicians who attend to and treat patients with the diseases to better understand the diversity of each of the patient populations to be studied and to obtain more restricted populations of patients (e.g., discordant monozygotic twins, drug-responsive versus nonresponsive cohorts, severity degrees of the disease). These kinds of collaborations between bench and bedside may not only lead to more targeted hypotheses but may also assist in decreasing the variability reported for in vitro modeling.

Improving Culture Conditions to Better Mimic the In Vivo Environment

While two-dimensional cell cultures have been fundamental to cell biology, drug discovery, and tissue engineering, they are unable to fully recapitulate the complex and dynamic three-dimensional (3D) environment of the tissue in vivo. Microfluidics technology allows an engineered platform for 3D cell culture with complex and dynamic microenvironments that are controllable and reproducible. Current approaches to reducing the variability in iPSC-disease models often utilize multiple iPSC clones derived from select cohorts of patients. Microfluidic devices fabricated from oxygen-permeable material such as polydimethylsiloxane (PDMS) can support long-term neural cultures while occupying less space and using significantly fewer reagents than traditional tissue-culture techniques, making it feasible to conduct experiments involving a large number of iPSC lines for disease modeling and drug screening. The microscale dimensions of the microchannel designs are comparable to in vivo cytoarchitectural features and can create multiple chemical gradients to simulate endogenous in vivo auto- and paracrine signaling cues. iPSC-based disease models have just begun to fully explore the possibilities offered by this technology. An interesting study demonstrating the precision and control of these devices differentiated human embryonic stem cells (hESCs) as embryoid bodies (EBs) on a Y channel device and was able to induce differentiation on half of a single EB while simultaneously maintaining the other half in an uninduced state (Fung et al., 2009). Similarly, maintenance of hESC self-renewal and differentiation can be manipulated at the single-colony level (Villa-Diaz et al., 2009).

In addition, micropatterning using biomaterials (i.e., collagen, laminin) combined with fabrication of physical structures allows for the isolation of dendrites and axons as well as compartmentalization of cellular subtypes to create highly organized structures that can mimic the organization of the endogenous tissue or organ (Figure 1). A study using 3D micropatterned neuronal cultures showed that chemical gradients of nerve growth factor (NGF) and the serum substitute, B27, could orient the direction of neurite outgrowth and regulate synapse distribution (Kunze et al., 2011a, 2011b). And finally, microfluidic platforms can integrate cell culture with subsequent cell-based assays such as genetic and protein analysis on a single device, providing a versatile tool for accurate quantification of biometrics that can be adapted for high-throughput, high-content screening.

Table 1. Neurological Syndromes for which iPSCs Have Been Derived

Disease	Genetic Defect	Neurological Symptoms	Phenotype in hiPSC-Derived Neural Progeny	Therapeutic Approach: Genetic Manipulation or Drug	Reference
Adrenoleukodystrophy	*ABCD1*	Demyelination and central and peripheric nervous system progressive loss of function	Very long chain fatty acid level was increased in oligodendrocytes	Lovastatin, 4-phenylbutyrate	(Jang et al., 2011)
Alzheimer's disease (AD)	Multifactorial or *PS1*, *PS2*, *APP* duplication	Progressive memory disorientation and impaired cognition	Increased amyloid β (Aβ) secretion, increased phospho-tau (Thrc231) and active glycogen synthase kinase-3β (aGSK-3β)	γ-secretase inhibitor decreased (Aβ) secretion β-secretase inhibitors reduced phospho-Tau (Thrc231) and aGSK-3β levels	(Yagi et al., 2011; Israel et al., 2012)
Amyotrophic lateral sclerosis (ALS)	*SOD1*, *VAPB*, *TDP43*	Neuromuscular degeneration and progressive loss of upper and lower motor neurons, causing weakness and paralysis	VAPB: reduced levels of VAPB in motor neurons derived from patients with VAPB mutation TDP43: mutant neurons had elevated levels of soluble and detergent-resistant TDP-43 protein, decreased survival in longitudinal studies, and increased vulnerability to antagonism of the PI3K pathway	N/A	(Dimos et al., 2008; Mitne-Neto et al., 2011; Egawa et al., 2012)
Huntington's disease (HD)	CAG repeat expansion in Huntingtin gene (*HTT*)	Progressive chorea and dementia associated with loss of striatal medium spiny neurons and cortical neurons	HD-neural stem cells showed susceptibility stress; vulnerability to BDNF withdrawn, increased cell death and altered mitochondria bioenergetics. Formation of protein aggregate inclusions after treatment with proteasome inhibitor (MG132). Vacuolation in HD-astrocytes. Increase in lysosomal activity in HD-iPS cells	Genetic correction by homologous recombination	(Zhang et al., 2010; An et al., 2012; Camnasio et al., 2012; Chae et al., 2012; HD iPSC Consortium, 2012)
Familial dysautonomia (FD)	*IKBKAP*	Degeneration of sensory and autonomic neurons	Decreased expression of genes involved in neurogenesis and neuronal differentiation; defects in neural crest migration	Kinetin	(Lee et al., 2009)
Parkinson's disease (PD)	*LRRK2*, *PINK1*, *SNCA* and Parkin	Age-related degeneration of both central and peripheral nervous systems	Impaired mitochondrial function in PINK1-mutated dopaminergic neurons; sensitivity to oxidative stress in LRRK2 and SNCA-mutant neurons. Reduced dopamine reuptake and increase of spontaneous dopamine release	N/A	(Devine et al., 2011; Nguyen et al., 2011; Seibler et al., 2011; Jiang et al., 2012; Peng et al., 2013)

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Table 1. ***Continued***

Disease	Genetic Defect	Neurological Symptoms	Phenotype in hiPSC-Derived Neural Progeny	Therapeutic Approach: Genetic Manipulation or Drug	Reference
Rett syndrome (RTT)	*MeCP2* *CDKL5*	Large spectrum of autistic characteristics, impaired motor function, regression of developmental skills, hypotonia, seizures; atypical Rett syndrome has clinical features closely related to Rett syndrome, including intellectual disability, early-onset intractable epilepsy starting before the age of 6 months, and autism	MeCP2: neuronal maturation defects, decreased synapse number, reduced number of spines, smaller cell soma size, and elevated LINE1 retrotransposition CDKL5: aberrant dendritic spines	Insulin growth factor 1(IGF1), gentamicin	(Marchetto et al., 2010; Muotri et al., 2010; Ananiev et al., 2011; Koch et al., 2011; Ricciardi et al., 2012; Weinacht et al., 2012)
Schizophrenia	Multifactorial	Neuropsychiatric disease characterized by hallucinations, delusions, and disorganized speech. Pathological hallmarks involve aberrant neurotransmitter signaling, reduced dendritic arborization, and impaired myelination	Diminished neuronal connectivity and decreased neurite number, PSD95 and glutamate receptor expression. Increase in extramitochondrial oxygen consumption and elevated levels of reactive oxygen species (ROS)	Loxapine, valporic acid	(Brennand et al., 2011; Paulsen et al., 2012; Pedrosa et al., 2011)
Spinal muscular atrophy (SMA)	*SMN1*	Selective loss of lower motor neurons resulting in muscle weakness and paralysis	Reduced size and number of SMA-mutant motor neurons	Valporic acid, tobramycin	(Ebert et al., 2009)
Timothy syndrome	*CACNA1C*	Long-QT syndrome Neurological defects, autistic characteristics	Decreased expression of genes that are expressed in lower cortical layers and in callosal projection neurons, abnormal expression of tyrosine hydroxylase and increased production of norepinephrine and dopamine, activity-dependent dendritic retraction	Roscovitine Expression of RGK protein, *Gem*.	(Paşca et al., 2011; Yazawa et al., 2011)
Machado-Joseph Disease	*MJD1* (*ATXN3*)	Dominantly inherited late-onset neurodegenerative disorder caused by expansion of polyglutamine (polyQ)-encoding CAG repeats in the *MJD1* gene	Excitation-induced ataxin-3 aggregation in differentiated neurons	Elimination of SDS-insoluble fraction by Calpain inhibitors (ALLN, calpeptin)	(Koch et al., 2011)
DOWN syndrome (DS)	Trisomy 21	Mental delay, early-onset Alzheimer's disease	Cortical neurons develop AD pathologies: secretion of the pathogenic peptide fragment amyloid-β42 (Aβ42) and formation of insoluble amyloid aggregates. Presence of hyperphosphorylated tau protein on cell bodies and dendrites	γ-secretase inhibitor decreased (Aβ) secretion	(Park et al., 2008; Shi et al., 2012)

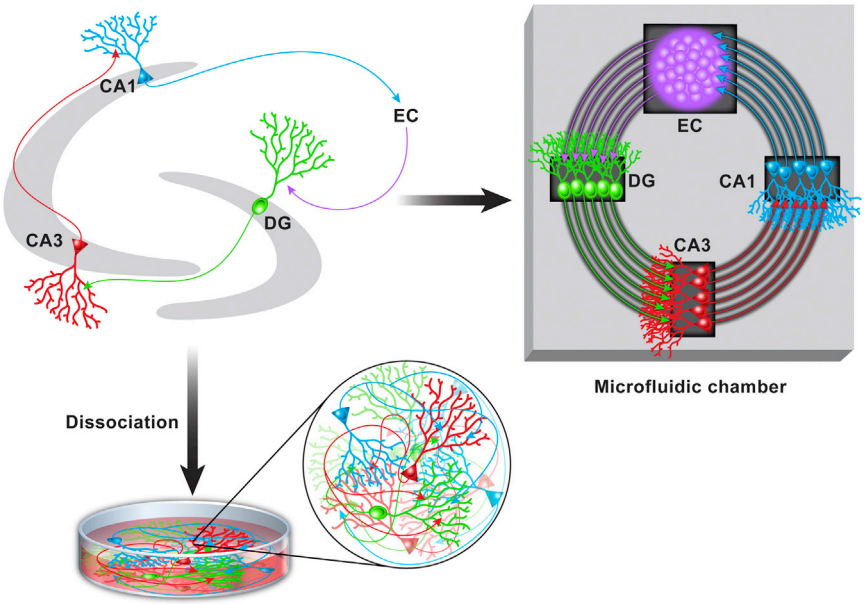

Figure 1. Proposed Use of Microfluidic Chambers for Proper Reproduction of Hippocampal Circuitry

Mircropatterning using biomaterials combined with of bioengineered cell chambers allow for isolation of dendrite and axons as well as compartmentalization of cellular subtypes to create highly organized structures that can mimic the organization of the endogenous tissue.

While engineering platforms allow the researcher precision and control over the cellular microenvironment, in vivo transplantation of stem cell-derived populations of human pluripotent stem cells (hPSCs) and neurons into animal models presents a useful way to study human development and model disease. Grafting the neural progenitor cells (NPCs) at appropriate developmental stages could potentially utilize the myriad biochemical and biophysical cues provided in the endogenous niches to generate mature and functional populations of the desired cells. An excellent example is the transplantation of hPSC-derived forebrain NPCs into the neonatal mouse brain to generate cortical neurons with specific axonal projections and dendritic patterns corresponding to the native cortical neuron population (Espuny-Camacho et al., 2013). These transplanted human cortical neurons showed progressive differentiation and connectivity over several months in vivo, demonstrating that these cells can develop properties characteristic of developmental corticogenesis and may offer opportunities for modeling of human cortex diseases and brain repair. In addition, transplantation of hPSC-derived medial ganglionic eminence (MGE) progenitors into the rodent brain produced GABAergic interneurons with mature physiological properties along an intrinsic timeline that mimics the endogenous human neural development (Maroof et al., 2013; Nicholas et al., 2013). As MGE-derived cortical interneuron deficiencies are implicated in a number of neurodevelopment and degenerative disorders, this technique may be used to model human neural development and disease. Finally, another still controversial alternative would be the use of human-mouse chimeras generated from hESC engraftment to mouse blastocysts (Siqueira da Fonseca et al., 2009); however, the extent to which these cells recapitulate human development remains to be determined.

Characterizing Neuronal Connectivity and Network Properties

A unique function of the nervous system is its dependence on properties that emerge from the networks of neurons and glia cells. While much research had been done looking at the cellular properties of its individual constituents (neurons or glia), we are just beginning to formulate the tools that would allow us to examine the emergent properties of these complex neural networks (Power et al., 2011). It is clear that neurodegenerative and psychiatric disorders, while exhibiting disease attributes at the single-cell level, are also manifestations of alterations in structure and function at the network level (Seeley et al., 2007; Church et al., 2009; Seeley et al., 2009). Recent work using iPSCs for disease modeling also demonstrated that there might be significant defects in the connectivity of neuronal networks of patients with autism and schizophrenia (Marchetto et al., 2010; Brennand et al., 2011). Substrate-integrated microelectrode arrays (MEAs) fabricated with semiconductor-based techniques can be a useful tool to further investigate the connectivity of functional neural networks. These platforms have been demonstrated to support long-term neuronal culture (Musick et al., 2009) and can be combined with microfluidics designs to record activity between distinct populations of neurons (Kanagasabapathi et al., 2011). Thus far in the field of iPSC research, MEAs have been mostly used with iPSC-derived cardiomyocytes to measure extracellular field potentials and has been combined with imaging modalities (i.e., intracellular calcium) to provide information about electrical coupling and action potential propagation between cells (Lee et al., 2012a).

The application of MEAs in neuroscience has been limited in part by the fact that, while it can simultaneously record multiple neurons and observe them over long periods of time, MEAs can only measure extracellular field potentials and cannot replace the full electrophysiological repertoire (subthreshold synaptic potentials, membrane oscillations, fast-spiking action potentials, etc.) offered by traditional intracellular recordings. However, recent advances in MEA technology are moving toward designs that can provide intracellular recording in addition to the traditional substrate-integrated MEA platforms. One promising design is the gold mushroom-shaped microelectrodes (gMμEs), which are shaped to mimic the dendritic spine and functionalized with extracelluar matrix (ECM) binding domains to facilitate endocytosis and cytoskeletal rearrangement around the microelectrode. Individual gMμEs can monitor action potentials (APs) and subthreshold synaptic potentials; they can also evoke APs without damaging the cell (Hai et al., 2010a, 2010b). In addition, silicon nanowires fabricated as the gate electrode of field-effect transistors (FET) and coated with phospholipids

have been demonstrated to spontaneously fuse with the plasma membrane and perform intracellular recordings of APs (Tian et al., 2010; Duan et al., 2012). While the long-term stability and modalities of these designs have to be further validated in primary and stem cell-derived neurons, they present very exciting possibilities for future developments in the iPSC field as tools in basic scientific research and drug discovery.

Translational and Clinical Opportunities for Pluripotent Stem Cells

Stem Cell-Based Platforms for Drug Discovery

While regeneration of diseased tissue to restore function remains the holy grail of stem cell therapy, a more immediate therapeutic role for iPSCs may be as a platform for drug discovery. Development of new drugs is an expensive and time-consuming process where ~90% of drug candidates fail at clinical trials due to issues of safety and efficacy. Preclinical studies largely based on cell lines and animal models are limited by their inability to fully recapitulate normal cellular function, the lack of disease-relevant functional assays, and interspecies differences in biological pathways as well as pharmacokinetic properties. iPSCs offer a number of advantages over the traditional methods. Disease-specific iPSCs can provide a renewable source of human cells with genetic background sensitive to disease pathology. A number of these iPSC-based disease models have demonstrated amelioration of disease phenotype in response to known therapeutic agents (Marchetto et al., 2010; Brennand et al., 2011; Israel et al., 2012). Drug screening using these cellular models could provide a more sensitive and accurate assessment of the test compounds. A recent study used iPSCs-derived dopaminergic neurons to screen a group of compounds for neuroprotective properties as a treatment strategy for early stages of PD. Of the 44 compounds that demonstrated therapeutic effects in rodent systems, only 16 provided significant neuroprotection in the rotenone-induced dopaminergic neuron cell death model for PD, emphasizing the importance of using disease-relevant human neurons for these assays (Peng et al., 2013). An in-depth discussion of using human pluoripotent stem cells to build more physiologically relevant in vitro assays for drug development is presented by Engle and Puppala (2013) in this issue.

Work is underway to develop high-throughput screening (HTS) assay systems to evaluate small molecule therapy for CNS diseases using iPSCs. To scale up from validating a few compounds to screening large chemical libraries, some key issues must be addressed. Aside from large-scale production of the disease-relevant cell types, it is critical to define relevant phenotypes suitable for automated HTS assays. Common modalities used for high-throughput platforms include imaging-based assessment of cell viability and function as well as quantification of gene expression and protein levels. A recent study reported screening 6,912 small molecule compounds on neural crest precursors derived from familial dysautonomia (FD) patient iPSCs. The authors employed a tiered approach that first detected rescued levels of wt-IKBKAP, the gene responsible for FD, with qPCR-PCR, then followed up the eight hit compounds with further validation in additional iPSC clones as well as using immune blots and migration assays (Lee et al., 2012b). While these results are promising, findings from iPSC-based disease models for a number of CNS diseases have also identified more complex phenotypes such as connectivity and synaptic defects (see sections on iPSC disease models); the challenge remains to formulate strategies to screen for these attributes in a high-throughput format.

Finally, iPSCs may also be used to assess developmental as well as cell-type-specific drug toxicities. Indeed, there are already commercially available hiPSC-derived hepatocytes, cardiomyocytes, and neural cells that may provide the basis for humanized assays to detect off-target activity and side-effects of drugs in a tissue-specific manner (Scott et al., 2013). By incorporating relevant functional assessments such as drug transporter activity in iPSC-generated hepatocytes, beating profiles of cardiomyocytes, and synaptic activity of neurons, one might unveil toxicity pathways that could not be observed in previous cellular models and improve the safety profiles of candidate drugs during their preclinical development.

Pluripotent Stem Cells for Transplantation Therapies

Stem cell therapy has been explored in clinical trials since the late 1980s using human fetal neural stem cell (fNSC) transplantation for a variety of CNS disorders including PD (Lindvall et al., 1990; Isacson et al., 2003; Lindvall and Björklund 2004; Mendez et al., 2005), HD (Philpott et al., 1997; Freeman et al., 2000; Bachoud-Lévi et al., 2006), and ALS (Glass et al., 2012), in which a phase I study to assess intraspinal injection of fNSCs has been recently initiated. However, results from these clinical studies have varied greatly between patients. While there were a few sporadic cases of improvements in cognitive and/or motor functions following the transplant procedures (Bachoud-Lévi et al., 2006), it remains largely unclear whether the benefits of these transplant therapies outweigh the risks associated with the requisite surgical procedures and the graft-induced complications. Among other concerns, the limited availability of fetal tissue presents a major challenge in standardizing the cells used for these transplant procedures. This limitation not only contributes to the variability of the outcomes, but also complicates the interpretation of these study results. Human ESCs and iPSCs can potentially circumvent these difficulties by providing a renewable source of disease-relevant cells to serve as an alternative to fetal neural tissue for transplantation. Here, we will focus on the recent developments and findings using human ESC- and iPSC-derived cells for transplantation in clinical therapeutics.

Clinical Studies Using hESC and iPSCs

Although it has been only seven years since the introduction of somatic reprogramming technology to generate iPSCs, there are already clinical studies underway for bringing iPSC-based cell therapy to patients. The Takahashi group at the RIKEN Center for Developmental Biology in Kobe, Japan, is proposing to treat a cohort of six patients with severe age-related macular degeneration (AMD), a condition where deterioration of photoreceptors results in vision loss in the central visual field, by using cells derived from patient-specific iPSCs. Takahashi, who previously reported the differentiation of ESCs and iPSCs to functioning rod photoreceptors (Homma et al., 2013), plans to transplant sheets of iPSC-derived retinal cells into the subretinal space of AMD patients to rescue and restore the pigmented epithelium. A similar study using hESCs was published last year, where two patients (one with dry age-related macular degeneration and one with Stargardt's macular dystrophy)

received injections of 50,000 hESC-derived retinal pigmented epithelium (RPE) cells into the subretinal space of each patient's eye (Schwartz et al., 2012). No hyperproliferation, abnormal growth, or immune-mediated transplant rejection was observed in either patient at 4 months after the surgeries. The investigators reported anatomical evidence of hESC-derived RPE survival and engraftment in the patient with Stargardt's macular dystrophy by spectral domain ocular coherence tomography and improvement in visual acuity from hand motions to counting fingers at postoperative week 2. The AMD patient also demonstrated some visual improvement from 20/500 at baseline to 20/320 by week 6, although there were also mild functional increases in the fellow eye, confounding this result.

Takahashi's current study, less tightly regulated than a formal clinical trial by the Japanese Ministry of Health and Welfare, cannot by itself lead to approval of a treatment for clinical use. However, if approved, it will be the first clinical demonstration of iPSCs for medical use and will, without doubt, impact the outlook regarding the safety and efficacy of iPSC-based cell therapy.

Immunogenicity of iPSCs and Related Challenges for Extending iPSCs to Clinical Use

Despite the promise of iPSCs as an autologous cell source for transplant therapy that would theoretically mitigate host immune rejection of the grafted cells, the immunogenicity of iPSCs is still a controversial topic. The controversy was sparked by a study in 2011 that reported an unexpected immune reaction triggered by teratomas generated from syngeneic iPSCs. A significantly higher rejection rate was reported with the iPSC-derived versus ESC-derived teratoma and was linked to aberrant expression of a number of tumor-related genes including *Hormad1* and *Zb16* (Zhao et al., 2011). Two recent reports have challenged these findings, showing that terminally differentiated cell types (endothelial cells, hepatocytes, and neurons) did not induce T cell responses either in culture or after tissue engraftment (Guha et al., 2013). Moreover, there was minimal immune reaction against the teratoma tissue derived from syngeneic iPSCs established using integration-free methods (Araki et al., 2013). It is conceivable that differences in the vector choice used for reprogramming in these studies, i.e., retroviral-, lentiviral-, and integration-free plasmids, may have contributed to the immunogenicity differences observed in the subsequent iPSC lines (Kaneko and Yamanaka, 2013). However, more importantly, these studies highlight how much is still unknown regarding the basic biology of reprogramming technology, knowledge that will be critical for iPSCs to be safely used in clinical settings. Which method should be considered the reprogramming vector of choice to generate clinical-grade iPSC lines? Should each patient-derived iPSC line be individually assessed for its tumorigenicity as well as its efficiency of producing the disease-relevant cell type needed for the treatment? How will the cost and labor needed for quality control impact the feasibility of establishing iPSCs as a standardized therapy? In an effort to address the safety concerns regarding iPSCs in clinical use, the biotechnology firm Advanced Cell Technology (ACT), in Santa Monica, California, is applying for FDA approval for a less ambitious clinical trial of injecting hiPSC-derived platelets as a potential treatment of coagulopathies (http://www.ipscell.com/2012/12/advanced-cell-technology-actc-announces-plan-to-make-ips-cell-derived-platelets-some-thoughts/). Platelets, lacking a nucleus, would reduce the risks for tumors and tumor-associated immune responses. But the challenge remains: for iPSC cell therapy to be applicable in the clinical setting, much more groundwork is needed to better understand the biology of these reprogrammed cells and their progenies.

Bridging Bench to Bedside

To successfully advance hESC- and iPSC-based cell therapy to clinical trials, a number of additional special considerations remain to be addressed. Developing clear benchmarks for assessing these issues in the preclinical stages will greatly facilitate the evaluation and interpretation of outcomes in future clinical trials.

Despite promising evidence of differentiation, maturation, and integration of the grafted cells into the endogenous neural circuitry in animal models (Lu et al., 2012; Ma et al., 2012; Nicholas et al., 2013), cells used for transplantation must be rigorously assessed for their proliferation potential as well as their fidelity in generating the desired cell type. Finding accurate biomarkers for cell sorting or engineering regulated suicide genes for inducible apoptosis may provide ways to select for the desired cells for use in transplantation. Targeted selection of the desired cells not only would reduce the risk of tumorgenesis in vivo, but would also allow more accurate formulation of the optimal cell dosage for the intended therapy and identify optimal treatment windows for clinical studies. In addition, while a number of preclinical studies have demonstrated functional improvement after transplantation of hESC- and hiPSC-derived neurons in animals models (Roy et al., 2006; Wernig et al., 2008; Hargus et al., 2010; Jiang et al., 2011; Lu et al., 2012; Ma et al., 2012), the mechanisms of the recovery, whether it is due to reconstruction of damaged neural circuitry or neurotrophic support, remain unclear. Elucidating the precise mechanisms of functional recovery is critical for designing human trials, specifically for the determination of the best time course for follow-ups after the procedure and the methods of evaluation for therapeutic efficacy, both of which will maximize knowledge gained from these trials.

Furthermore, a critical factor for the success of hematopoietic stem cell transplantation, the only stem cell-based therapy globally accepted in the clinical setting, is the intrinsic ability of hematopoietic stem cells to home to the bone marrow. The mobility and migration potential of hESC/hiPSCs and their progenies have yet to be assessed in detail in vivo. The ability of transplanted cells to target the sites of disease and injury will greatly impact the types of conditions that are suitable for hESC/hiPSC-based cell therapy and will affect the surgical methods for delivering the transplants. Finally, the efficacy of hESC/hiPSC cell therapy should be compared with the current gold standard of treatment for the disease. Patients can have higher risk-tolerance toward experimental medicine; therefore, evidence of superior performance should be reproducibly established prior to movement into human trials.

In Vivo Reprogramming in Human Subjects

An attractive alternative to cell-replacement therapy would be to mobilize resident cells already present in the target tissue to repair the damage. One possibility would be to use on-site reprogramming technology to generate specific subtypes of cells that have been lost through aging, injury, or disease. A few successful attempts have been made to reprogram (or

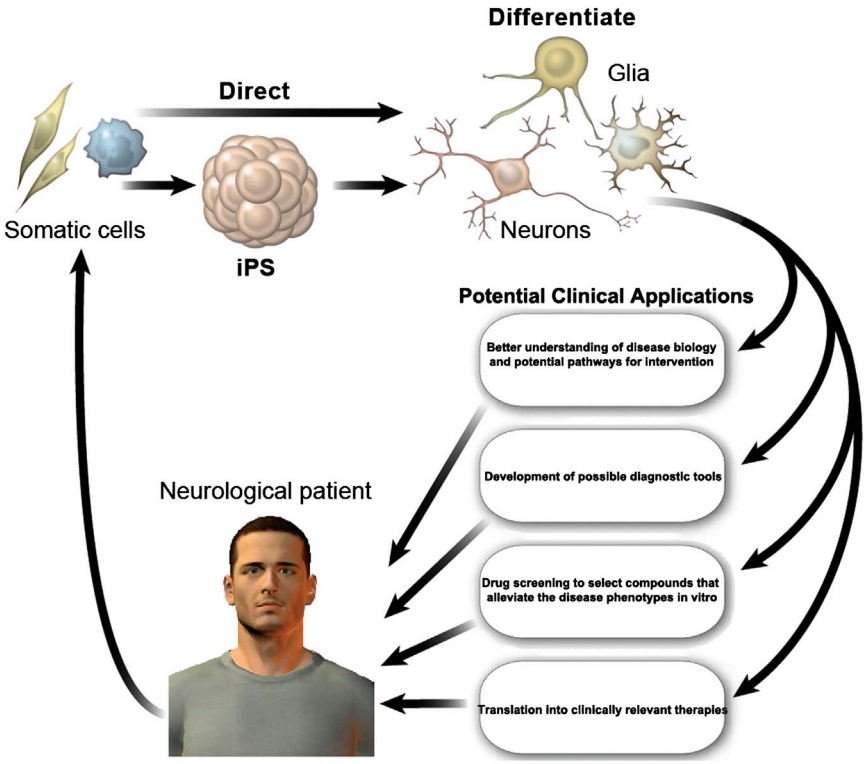

Figure 2. Potential Applications of Reprogramming Technology in the Clinical Setting for Neurological Diseases

Promising approaches include better understanding of disease biology, development of new diagnostic tools, formulation of new therapies, and personalized clinical interventions.

transdifferentiate) cells in the rodent central nervous system by ectopically expressing region-specific transcription factors (Jessberger et al., 2008; López-Bendito and Arlotta, 2012). In a recent study, transplanted human fibroblasts and human astrocytes engineered to express inducible forms of neural reprogramming genes (complex-like 1 [Ascl1], brain-2 [Brn2a], and myelin transcription factor-like 1 [Myt1l] converted into neurons after activation of these genes in vivo. Additionally, endogenous astrocytes in a transgenic mouse model with directed expression of these reprogramming genes to the parenchymal astrocytes in the striatum can be directly converted into neural nuclei (NeuN)-expressing neurons in situ (Torper et al., 2013).

There are currently a number of obstacles to be overcome before in vivo reprogramming in the nervous system becomes an accepted therapy for human neural pathology. The main challenges are identifying the cell types that are able to be reprogrammed in vivo and optimizing the methods of specific delivery of the reprogramming vehicle. Defining and targeting the best cell type in the nervous system will require basic knowledge of brain niche biology and dynamics. Examples of this work are underway but it will be critical to determine that reprogrammed cells are not only functional in vivo, but also are appropriately functional for the target areas or damaged circuit. This will require functional studies that demonstrate reprogramming and functional recovery and confirmation that the recovery depends on the reprogrammed cells. The replacement of the exact cell that is lost through disease or damage may not be necessary; it would be impressive enough that the reprogrammed substitution or compensatory mechanism caused functional recovery. In vivo reprogramming technology in the nervous system has the potential to become an important tool for generating significant therapies that are patient tailored, but a lot of fundamental research remains to be performed.

Conclusion/Future directions

Reprogramming technology has resulted in fundamental changes in how we think about cell biology, stimulating a rapid movement to clinical and commercial applications (Figure 2). We present here our perspective on this movement, suggesting that there are many positive developments that can occur. For modeling human disease and HTS using hPSCs, the risks are that the high variability in the methods and relative paucity of lineage-specific differentiation protocols may limit our ability to mimic or detect disease-specific phenotypic changes. The good news is that, with appropriate cell banking, iPSCs can allow multiple attempts on the same cohorts for discovery and screening. It is also encouraging to consider the engineering, chemistry, and material science advances that can be applied to optimize these in vitro studies.

The in vivo applications for cell replacement and endogenous reprogramming are still at very early stages of development. However, in some instances, thoughtful attempts at cell therapy using reprogramming technology are underway. Of course, the risk here is that failure will have greater consequences. Other cutting-edge areas, such as gene therapy, have suffered tremendously from just a single poorly implemented clinical trial. Even more disturbing is the current extent of unsubstantiated stem cell therapy offerings with little or no evidence for claims of efficacy. There is a need for concerted efforts to regulate stem cell clinical offerings by unscrupulous commercial enterprises globally. To this end, it is important to support the best and most carefully designed clinical studies, setting the bar high for what is expected for a successful clinical trial outcome.

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