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REVIEW

Application of reprogrammed patient cells to investigate the etiology of neurological and psychiatric disorders

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Abstract **Cellular reprogramming allows for the *de novo* generation of human neurons and glial cells from patients with neurological and psychiatric disorders. Crucially, this technology preserves the genome of the donor individual and thus provides a unique opportunity for systematic investigation of genetic influences on neuronal pathophysiology. Although direct reprogramming of adult somatic cells to neurons is now possible, the majority of recent studies have used induced pluripotent stem cells (iPSCs) derived from patient fibroblasts to generate neural progenitors that can be differentiated to specific neural cell types. Investigations of monogenic diseases have established proof-of-principle for many aspects of cellular disease modeling, including targeted differentiation of neuronal populations and rescue of phenotypes in patient iPSC lines. Refinement of protocols to allow for efficient generation of iPSC lines from large patient cohorts may reveal common functional pathology and genetic interactions in diseases with a polygenic basis. We review several recent studies that illustrate the utility of iPSC-based cellular models of neurodevelopmental and neurodegenerative disorders to identify novel phenotypes and therapeutic approaches.**

Keywords reprogramming, iPSCs, neurodevelopment, neurodegeneration

Introduction

One of the most promising technological advances in the field of biology has been the recent discovery that somatic cells can be reprogrammed to a state of pluripotency (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008). These induced pluripotent stem cells, or iPSCs, were first generated through the retroviral-mediated introduction of four key transcription factors, Oct3/4, Sox2, c-MYC and Klf4, into mouse embryonic or adult fibroblasts. Expression of these factors in fibroblasts induced upregulation of pluripotency markers and the capacity to differentiate into cell types from all three germ layers (Takahashi and Yamanaka, 2006). Using this same technique, it was later demonstrated that human fibroblasts could also be reprogrammed into iPSCs with characteristic properties of both long-term self-renewal and pluripotent differentiation capacity (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008).

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Perhaps the most salient feature of this technology for understanding human neurological diseases is that cell populations derived from iPSCs retain the genetic information of the donor. This affords a remarkable opportunity to track the development and monitor the function of human neurons in a disease-relevant genetic context. More recently, additional protocols for iPSC induction have been optimized that rely on different combinations of reprogramming factors, integration-free approaches to ensure an intact host genome, and even the direct application of small molecules to elicit a pluripotent state (Juopperi et al., 2011). In this review, we will highlight several recent studies that demonstrate the feasibility of using patient-specific iPSCs for quantitative analysis of phenotypic disturbances in human neurons (Fig. 1). We will also discuss the most significant challenges that lie ahead in the application of this technology for efficacious clinical intervention.

Neurodevelopmental disorders

Neurodevelopmental disorders are particularly challenging to model because the emergence of functional brain systems

depends on local growth signals and connectivity as well as the effective targeting of and feedback from distal structures (Uhlhaas and Singer, 2010). Brain development is in many ways a holistic process that requires the global integrity of all component systems. Even though systems may come "on-line" in a sequential manner, each stage depends on the success of the previous one. Cortical development, for instance, proceeds in an "inside-out" fashion, with more recently born neurons migrating past existing neurons residing in the deeper layers, and this migratory process requires the preexisting structural scaffolding of radial glial cells and extant guidance cues from neurotrophic factors and secreted proteins to reach the proper point of termination (Shi et al., 2012). In addition, functionally modular neural systems may be widely distributed within the brain, encompassing anatomically discrete loci and multiple neuronal subtypes. Therefore, small perturbations in local activity-dependent processes that are ubiquitous during early development can lead to global deficits in connectivity (Spitzer, 2006). For many neurodevelopmental disorders, it is difficult to identify the causal pathology that triggers aberrant brain development because the ancillary consequences of an initial imbalance can be so pervasive. This difficulty is compounded by the limitations of traditional approaches to investigate human neural pathology, which has been confined to functional imaging, EEG (electroencephalography) and MEG (magnetoencephalography) recordings of brain activity, genetic association studies, and post-mortem analyses of brain tissue, none of which can directly address the underlying mechanisms of disease etiology. The advent of stem cell technology provides an unprecedented opportunity to observe the development of human neurons in a system that is amenable to rigorous analysis of cellular function and genetic manipulation.

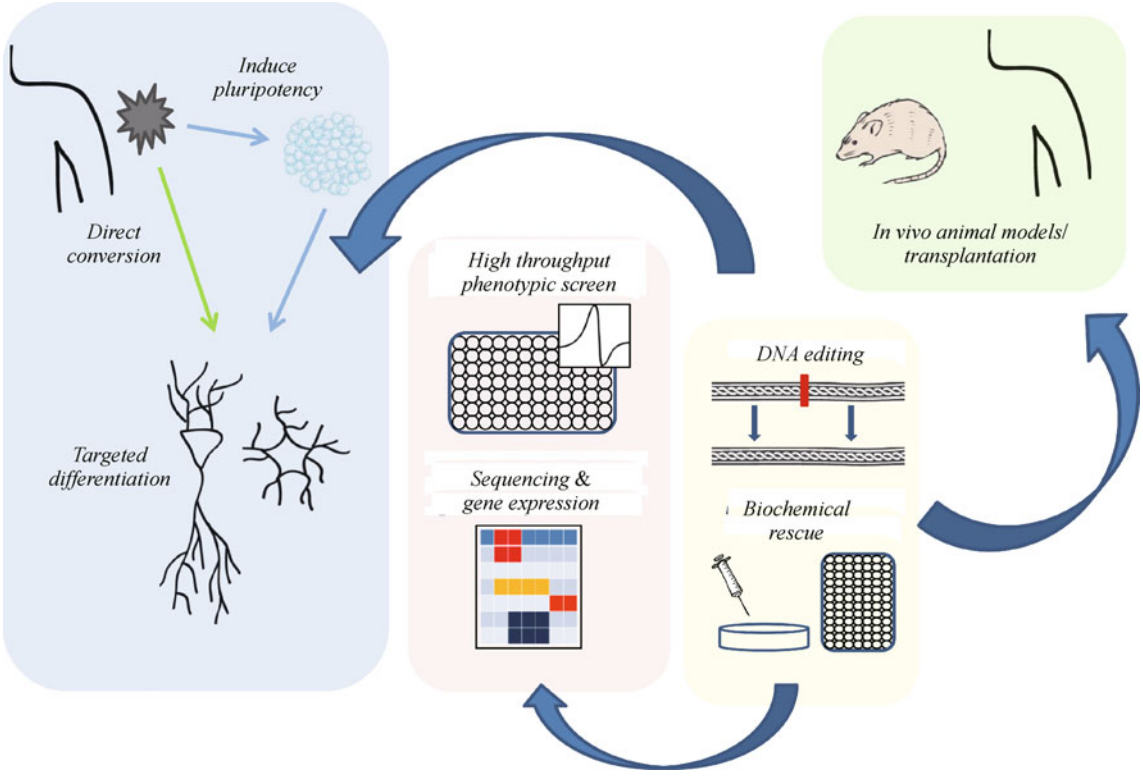

Figure 1 Simplified schematic diagram showing the development of cell-based assays for the investigation of neurological disease mechanisms. (Blue-shaded box) Using fibroblasts obtained from patients and disease-free control subjects, human neurons can be generated through direct conversion or following an intervening stage of pluripotency. Disease-relevant neuronal populations can be enriched during the differentiation process through targeted protocols. (Pink-shaded box) Phenotypic analysis of human neurons generated from patients and control subjects may include any cell-based morphological or functional assay (e.g. dendritic development, synaptogenesis, synaptic connectivity, electrophysiology, intracellular signaling) as well as genetic and epigenetic profiling to produce complete transcriptomes and methylomes of homogenous populations. High-throughput approaches are particularly desirable for polygenic diseases to identify common functional disruptions across a heterogeneous patient population. (Yellow shaded box) Based on information acquired during the phenotypic screens or from previous investigations, targeted genetic manipulation can repair known mutations, which can then be subjected to phenotypic screens or introduced to neural progenitor populations to track development following genetic repair. Bioactive compounds can be similarly screened at various timepoints to evaluate potential to reverse or prevent phenotypic abnormalities. (Green-shaded box) Transplantation of patient-derived neural progenitors to mice can provide critical information regarding development and degeneration *in vivo*. Ultimately, cell-based screening and repair of neuronal dysfunction can be used to develop novel therapeutics for the patient population.

Monogenic disorders

Perhaps the most immediately tractable neurodevelopmental diseases are those for which mutations in a single gene are highly or completely penetrant. These monogenic diseases have a discernible genetic cause, but for many of these disorders there is still a mechanistic gap in understanding how the gene variants induce disease symptomatology. However, the ability to focus on a single genetic locus narrows the investigative landscape and provides an ideal system in which to develop the methodology for evaluating cellular phenotypes, dissecting the relevant molecular pathways, and identifying strategies for genetic or biochemically-mediated rescue.

Rett syndrome

Rett syndrome (RTT) is a rare example of a monogenic autism spectrum disorder (ASD; Table 1). RTT is a neurodevelopmental disorder in which more than 95% of cases are due to mutations in the X chromosome-linked *MECP2* gene (Amir et al., 1999). Because *MECP2* resides on the X chromosome, males with *MECP2* mutations are prone to early lethality and females exhibit a wide range in the severity of symptoms due to the mosaicism of X chromosome inactivation. Thus, despite the monogenic basis of this disease, the X chromosome locus confers heterogeneity with respect to expression of MECP2 in cells of patients. A critical issue for cellular models of X-linked diseases is the degree to which iPSC lines are subject to reactivation of silenced X chromosomes. This has been controversial in recent years with some groups reporting that human iPSC lines, unlike reprogrammed cells from mouse fibroblasts, do not undergo X chromosome reactivation during reprogramming (Pomp et al., 2011; Tchieu et al., 2010), whereas others suggest that differentiated neurons can exhibit random inactivation (Marchetto et al., 2010). Interestingly, extensive passaging of iPSCs results in the apparent loss of the large

Table 1 Representative patient-specific iPSC studies of neurological disorders

Disease	Mutation	Differentiation	Phenotype	Rescue	References
RTT	*MeCP2* missense, nonsense and frameshift mutations	GABAergic and glutamatergic neurons	Reduced soma size, number of spines, glutamatergic synapses; altered Ca^{2+} transients, sEPSCs, sIPSCs	IGF1 – partial increase in synapse number; Gentamycin — restored MeCP2 expression in nonsense mutation	Marchetto et al., 2010
TS	*CACNA1C* point mutation	Layer-specific cortical neurons	Differential gene expression; TH expression	~	Paşca et al., 2011
FD	*IKBKAP* point mutation	CNS and PNS precursors	*IKBKAP* splicing, neurogenesis, migration of neural crest precursors	Kinetin rescue of splicing and autonomic neuron differentiation	Lee et al., 2009
SZ	4bp deletion in *DISC1*-frameshift mutation	~	~	~	Chiang et al., 2011
SZ	Not known	Glutamatergic, GABAergic and dopaminergic neurons	Decreased neuronal connectivity, increased *NRG1* expression	Loxapine rescue of neuronal connectivity deficits, *NRG1* expression	Brennand et al., 2011
PD	*LRRK2*dominant missense mutation	Midbrain dopaminergic neurons	Differential gene expression; increased α-synuclein expression, increased susceptibility to H_2O_2, 6-OHDA, and MG-132	~	Nguyen et al., 2011
PD	*PINK1* nonsense or missense mutations	Dopaminergic neurons	Impaired stress-induced translocation of Parkin to mitochondria; increased PGC-1α and mtDNA following depolarization	Overexpression of WT *PINK1* restored translocation capacity and prevented PGC-1α increase	Seibler et al., 2011
PD	*PARKIN* exon deletion (3 and/or 5)	Midbrain dopaminergic neurons	Increased spontaneous dopamine release; enhanced transcription of MAO-A, MAO-B; increased oxidative stress	Overexpression of WT-parkin rescued all phenotypes	Jiang et al., 2012
PD	α *synuclein* point mutation	Dopaminergic neurons	~	ZFN gene-editing; repair of point mutation in patient iPSCs; introduction of point mutation in hESCs	Soldner et al., 2011
AD	*APP* duplication in 2 patients (APP^{Dp}); No identified mutations in 2 sporadic AD patients (sAD)	Glutamatergic, GABAergic and cholinergic neurons	Increased amyloid-β, p-tau, and aGSK-3β expression in both APP^{Dp} lines and 1 of 2 sAD lines	Partial rescue of amyloid-β, p-tau, and aGSK-3β expression with γ and β-secretase inhibitors	Israel et al., 2012

Abbreviations: RTT, Rett syndrome; FD, familial dysautonomia; TS, Timothy syndrome; SZ, schizophrenia; PD, Parkinson's disease; AD, Alzheimer's disease; TH, tyrosine hydroxylase; MAO-A, monoamine oxidase A; MAO-B, monoamine oxidase-B; ZFN, zinc finger nuclease; mtDNA, mitochondrial DNA.

non-coding RNA, *Xist*, which is generally considered a signature of inactivation and which may account for some of the discrepancy in results. Non-random retention of X-chromosome inactivation in clonal progeny of iPSCs would allow for the analysis of homogenous populations of differentiated neurons with identified mutations in the *MECP2* gene derived from female RTT patients. Although this property is advantageous in early screening of phenotypic deficits, more advanced cellular models of disease would benefit from random X-inactivation to observe non-cell autonomous or network effects resulting from heterogenous neuronal populations that recapitulate conditions in RTT patients. When using fibroblasts derived from female patients, targeted sequencing and reliable indicators of X-inactivation will be necessary to characterize the neuronal population under investigation in studies of X-linked disease mechanisms.

One of the most extensive characterizations of cellular phenotypes in neurons derived from female RTT patients comes from a study in which the resultant population of cells was described to exhibit heterogeneity in X-inactivation at the cellular level. Nevertheless, specific phenotypes were observed in all differentiated neurons derived from patient fibroblasts when compared to neurons derived from fibroblasts of disease-free control subjects – namely, morphological defects in soma size, spine density and synapse number and electrophysiological defects in calcium signaling and evoked activity (Marchetto et al., 2010). To evaluate different pharmaceutical rescue strategies, the authors first investigated the effect of IGF1, a nonspecific growth factor, and observed a partial rescue of the phenotype through an increase in synapse number, mirroring previous results observed following systemic IGF treatment in a mouse model of RTT (Tropea et al., 2009). Importantly, some of the observed neuronal morphological defects were confirmed in independent studies (Cheung et al., 2011; Kim et al., 2011). Collectively, these studies suggest the existence of non-cell autonomous effects of MECP2 deficiency. In a majority of RTT patients, MECP2 expression is disrupted through a nonsense mutation. To enhance MeCP2 expression directly, the effect of gentamicin, an antibiotic that impairs ribosomal proofreading, was evaluated and shown to be capable of not only elevating MECP2 expression but also restoring the functional property of glutamatergic synaptic transmission. Although these pharmacological approaches need to be refined and extended, these functional rescue experiments demonstrated the feasibility of using disease-specific iPSC lines to identify putative strategies for clinical intervention.

Timothy syndrome

Timothy syndrome (TS) is an extremely rare, pervasive developmental disorder that affects both cardiac function and the central nervous system. All known cases appear to be caused by a single point mutation in the *CACNA1C* gene which encodes the α_1 subunit of the L-type calcium channel, $Ca_V1.2$. Most patients diagnosed with TS also meet the criteria for an autism spectrum disorder. The fact that a known functional mutation leads to behavioral symptoms reflective of ASD suggests that a deeper understanding of this rare disorder may reveal general properties governing developmental dysregulation leading to autistic-like cognitive symptoms. A recent comprehensive study evaluated several facets of neurodevelopment arising from TS patient iPSCs (Paşca et al., 2011). Direct effects of the mutant form of $Ca_V1.2$ include delayed inactivation of the channel and a sustained increase in intracellular calcium. Intracellular calcium signaling through L-type voltage gated channels is known to be associated with activity-dependent gene expression. Indeed, between control and TS neurons, several genes that showed differential expression were associated with the transcription factor, cAMP response-element binding (CREB) and its downstream targets. Other differentially expressed genes were associated with dopaminergic and norepinephrine signaling, including tyrosine hydroxylase (TH), the rate-limiting enzyme necessary for dopamine and norepinephrine production. Among the most prominent phenotypes observed at the population level was a differentiation bias toward upper layer cortical neurons derived from TS-iPSCs, which suggests that there may be a relative increase in the production of subcortical projection neurons at the expense of collosal projections. Importantly, this close analysis of specific neuronal subtypes revealed a potential imbalance in the composition of cortical neurons that could have a distributed impact across many functional domains and may warrant investigation in other forms of ASDs.

Familial dysautonomia

Familial dysautonomia (FD) is a rare disease predominantly arising from a point mutation in the I-k-B kinase complex-associated protein (*IKBKAP*) gene, which results in severe autonomic nervous system dysfunction and respiratory, gastrointestinal and cardiovascular problems. Previous reports had indicated tissue-specific splice expression of the *IKBKAP* gene in FD patients (Slaugenhaupt et al., 2001). Taking advantage of the ability to differentiate iPSC lines into multiple cell types, including CNS and PNS precursors, hematopoietic, endothelial and endoderm cells, a recent study was able to observe to some degree the differential splicing in cell types derived from patient-specific iPSCs, although full characterization of splice-variant induced suppression of IKBKAP in neural crest lineages is still needed (Lee et al., 2009). In this same study, the development of neurons from neural crest precursors generated from patient iPSCs was significantly delayed compared to neurons derived from control subjects. Earlier studies of lymphoblast cell lines from FD patients had identified several candidate compounds that were capable of effectively restoring IKBKAP expression (Anderson et al., 2003a; Anderson et al., 2003b; Slaugen-

haupt et al., 2003). By screening this small set of compounds with patient-derived iPSCs, it was shown that kinetin, a plant hormone, was capable of preventing splice variation and deficits in autonomic neuronal development (Lee et al., 2009).

Polygenic disorders

For most neurodevelopmental disorders, including the vast majority of ASD and schizophrenia, no single gene has been shown to be causally sufficient to induce disease symptomatology. In genome-wide association studies, several hundred genes have emerged as candidate "risk" genes for disease occurrence but there are contextual factors that may contribute to whether a given individual who harbors one of these identified mutations will eventually exhibit clinical symptoms (Keller and Persico, 2003; Harrison and Weinberger, 2005; Herbert, 2010). One such factor is the interaction of genetically-mediated risk and environmental stressors. Another is epistatic or synergistic interactions among risk genes. A third, non-exclusive, possibility is long-lasting modification of gene expression through epigenetic regulation. In addition, there are little data on how individual risk genes may alter cellular function in the absence of compounding factors and whether vulnerability to subsequent contextual influences is detectable on a mechanistic level. This very complexity highlights the potential utility of using cellular models to investigate these various possibilities.

Schizophrenia

Despite the delayed onset of schizophrenia (SZ), which typically occurs in late adolescence, the pathophysiology underlying this disease is considered to be developmentally regulated (Weinberger, 1987). There is no single genetic marker of disease that has shown high penetrance across patient populations, but several genes have been associated with increased susceptibility in multiple families. One such gene is Disrupted-in-Schizophrenia 1 (*DISC1*), which has also been shown to play a critical role in neuronal development (St Clair et al., 1990; Millar et al., 2000; Ross et al., 2006; Duan et al., 2007; Kim et al., 2009; Mao et al., 2009). Although mutations in DISC1 alone cannot fully explain the preponderance of SZ cases, investigation of its function has proven to be an exemplar for understanding complex genetic disorders and the potential for increased risk due to gene interactions and environmental influences. Much of the insight into the function of DISC1 and its potential role in SZ comes from complementary studies of this gene in early cortical development and in adult neurogenesis in rodents. In adult neurogenesis, knockdown of DISC1 leads to accelerated neuronal development in the form of somatic hypertrophy, excessive growth of axonal and dendritic processes, aberrant migration beyond the granule cell layer and premature synaptic integration (Duan et al., 2007; Faulkner et al., 2008). But the function of human DISC1 and how its disruption might contribute to the SZ pathology remains elusive. To evaluate potential genetic interactions and signaling pathways related to DISC1, several iPSC lines were derived from an American family in which a 4 base-pair deletion in *DISC1* associates with increased risk for SZ and major depressive disorders (Sachs et al., 2005; Chiang et al., 2011). Demonstrating the success of an episomal vector approach for integration-free reprogramming, the iPSCs were confirmed to exhibit the same 4bp frameshift mutation in DISC1 and provide a platform for comparing the phenotypic effects of human vs. mouse DISC1 expression in neuronal development.

Another recent study generated iPSC lines from SZ patients with strong family histories of mental illness but no identified specific genetic link among them (Brennand et al., 2011). Neurons differentiated from the patient iPSCs had deficits in connectivity, which were partly rescued by administration of the antipsychotic drug, loxapine, during neuronal differentiation. Despite decreased levels of some synaptic proteins, functional synaptic transmission appeared to be normal in the patient-derived neurons. RNA expression analysis revealed putative genetic associations among the patients in the form of altered expression several genes, including *NRG1* and *ANK3*, which were common to all neurons derived from the patient-specific iPSCs. Although this study involved only a small heterogeneous cohort of SZ patients, on a larger scale this approach to evaluate gene expression in an unbiased screen may lead to the identification of novel genetic interactions and key signaling pathways that are disrupted in neurons derived from unrelated patient populations. For polygenic diseases, the development of targeted and effective therapeutic strategies will be greatly facilitated by identifying whether there is a common pathway or functional disturbance that can be centrally addressed and associated with multiple genetic risk factors.

Neurodegenerative disorders

In some ways, strictly segregating neurodevelopmental disorders from neurodegenerative diseases may prove to be an oversimplification. The delayed onset of neurodevelopmental disorders such as SZ and RTT syndrome, illustrate the possibility of relatively long-term cellular vulnerability that precedes disease onset. Similarly, the gradual loss of neuronal populations in the mature brain may be preceded by a prolonged period of cellular instability and genetically conferred susceptibility to neurodegenerative disorders may become manifest long before any behavioral symptoms can be observed. Nevertheless, for many degenerative diseases that onset late in life, pervasive developmental dysregulation during early critical periods of brain development is unlikely. For this reason, there are different challenges that apply to modeling these disorders in cell-based systems. Perhaps most

critical is the need to determine how cellular age impacts the onset of degeneration and how to recapitulate aging-associated properties efficiently in neurons that have been recently developed from stem cells.

Parkinson's disease

Parkinson's disease (PD) is marked by the specific loss of dopaminergic neurons in the substantia nigra and typically onsets during middle age resulting in motor dysfunction and difficulty with movement initiation and termination. PD pathogenesis has been associated with several genes including *SNCA* (α-*synuclein)*, *LRRK2*, *PTEN-induced putative kinase 1* (*PINK1*), *PARK2 (PARKIN)*, and *DJ-1* (Martin et al., 2011). Cellular reprogramming of fibroblasts from PD patients is an emergent focus of stem cell technology and comparative data on the function of these genes in human neurons is beginning to coalesce. In an iPSC line generated from a PD patient with a *LRRK2* mutation, iPSC derived dopaminergic neurons, the most affected neuronal population in PD, exhibited a selective vulnerability to oxidative stress (Nguyen et al., 2011). On the other hand, in iPSCs derived from patients with a *PINK1* mutation, dopaminergic neurons were impaired in induced mitochrondrial translocation of PARKIN and an accumulation of mitochondrial DNA was observed that was due to either impaired clearance of dysfunctional mitochondria or increased production following neuronal depolarization (Seibler et al., 2011). Recently, targeted differentiation of midbrain dopaminergic neurons was achieved from iPSCs derived from PD patients with a mutation in the *PARKIN* gene (Jiang et al., 2012). In these cells with a functional loss of PARKIN, no changes in mitochondrial DNA levels were observed, but monoamine oxidase transcript levels were increased. In addition, dopaminergic signaling was altered due to an increase in spontaneous release and a suppression of dopamine uptake. Because monoamine oxidase function has been implicated in dopamine-induced oxidative stress, these findings suggest a mechanistic link that could reveal new targets for therapeutic rescue of this population. The studies with PD iPSCs highlight the differential cellular function of PD genes, whether these functions converge onto common pathways in contributing to the cell death of dopaminergic neurons in PD patients remains to be examined.

Because late-onset neurodegenerative diseases may show more subtle phenotypes during early stages of development and differentiation, variability in genetic background of iPSC lines may make identification of disease-relevant deficits more difficult. To address this issue, zinc-finger-nuclease (ZFN) gene editing was used in a recent study to create isogenic stem cell lines that differed in only two point mutations within the PD associated α-*synuclein* gene (Soldner et al., 2011). ZFN editing relies on combining specific DNA binding domains with a generic cleavage domain to target restricted regions of interest in the genome. Double strand breaks can then either be repaired through non-homologous end-joining or through homologous recombination to insert desired sequences. Using the ZFN strategy, both with and without exogenous selection markers, these authors demonstrated the ability to introduce point mutations into the α-synuclein gene of embryonic stem cell lines as well as the ability to repair mutations in patient-derived iPSCs via wild-type base substitution. This highly selective genetic manipulation allows for the generation of neurons with isogenic backgrounds that vary only in putative disease-causing mutations. For diseases such as PD, this approach may be pivotal to the potential development of therapeutic cell replacement strategies.

Alzheimer's disease

In contrast to the spatially restricted degeneration associated with a primary cell type in PD, Alzheimer's disease (AD) is characterized by the presence of amyloid plaques and neurofibrillary tangles and the potential degradation of neurons in several cortical and subcortical regions. In a recent study, iPSC lines were generated from two patients with sporadic AD and two with familial AD, caused by a duplication in the amyloid-β precursor protein gene (*APP*) that is associated with the accumulation of amyloid plaques (Israel et al., 2012). Neurons from both patients with familial AD and one patient with sporadic AD exhibited higher levels of amyloid-β when compared to neurons derived from control subjects with no age-related dementia. Interestingly, lines from these three patients also showed higher relative levels of phosphorylated tau, and increased GSK-3β activity, which was partially rescued following application of β-secretase inhibitors. This suggests a putative mechanistic relationship between plaque and tangle formation. Further, these results indicate the existence of genetic variants that have a phenotypic similarity to *APP* mutations in a subset of sporadic AD cases. In contrast, neurons derived from the second patient with sporadic AD shared none of these phenotypes. This finding itself is important and demonstrates the need to determine whether there is variable developmental latency in the emergence of stereotypical phenotypes or fundamentally different mechanisms that account for some forms of sporadic AD.

Future directions

Direct programming

Although reliable differentiation of neurons has been demonstrated from iPSCs and remains the primary means of generating functional neuronal subtypes from patient-specific fibroblasts, the ability to directly convert fibroblasts to neurons is an emerging technology with several distinct advantages (Vierbuchen et al., 2010; Ambasudhan et al.,

2011; Caiazzo et al., 2011; Pang et al., 2011; Pfisterer et al. 2011; Qiang et al., 2011; Yoo et al., 2011). For neurodegenerative disease, the possibility that directly converted neurons may retain age-specific properties of the donor fibroblasts may be beneficial to study the interaction between cellular age and vulnerability to disease (Chambers and Studer, 2011). For diseases with a neurodevelopmental origin, the ability to rapidly generate neuronal populations may expedite phenotypic analysis and facilitate high-throughput screening. Generating human neurons without the intervening stage of pluripotency may also minimize the risk of tumorigenesis and the retention of epigenetic modifications that are associated with pluripotency. Much work remains to be done to characterize the residual markers of the source cells and the process employed to generate human neurons. This will be critical to the development of cell replacement and tissue engineering strategies using reprogramming technology (Gore et al., 2011; Hussein et al., 2011; Lister et al., 2011).

Differentiation into specific neuronal subtypes

A central issue when considering the utility of patient-specific iPSCs to model features of disease etiology is the targeted differentiation of pluripotent cells to the most relevant cell types. Many differentiation protocols and individual iPSC clones appear to be intrinsically biased toward the development of a particular cell type for reasons that are not fully understood (Bock et al., 2011; Boulting et al., 2011). However, rapid progress is being made to direct cell-specific differentiation and identify the factors that enable enrichment of specific populations including dopaminergic, GABAergic, glutamatergic and layer-specific cortical neurons, as well as glial subtypes (Caiazzo et al., 2011; Hansen et al., 2011; Krencik et al., 2011; Paşca et al., 2011). For diseases such as schizophrenia, long-standing hypotheses regarding dysregulation of inhibitory circuitry mediated by GABAergic interneurons or an imbalance in dopaminergic signaling suggest primary cellular targets for exploration of developmental dysfunction. But a focus on cell-autonomous pathology should be balanced by the recognition that neural systems are interdependent and developmental perturbations in a particular neuronal subtype may lead to morphological and synaptic disturbances that can affect both local circuitry and distributed neural networks. This is a critical challenge for the investigation of developmental pathology using cellular model systems and a future goal should be to establish intermediate cellular networks that comprise multiple neuronal cell types and glia that are patterned on known properties of *in vivo* networks.

High-throughput phenotyping and drug-screening

The ability to generate human neurons in a rapid and efficient manner is essential for the large-scale screening of bioactive compounds that may ameliorate identified phenotypes in cell populations. While the generation of multiple iPSC lines and step-wise neuronal differentiation is still a labor-intensive process, further refinement of protocols to increase efficiency and the advent of direct reprogramming may soon allow for a significant increase in our capacity to generate multiple lines from larger populations of patients with similar clinical diagnoses. Identification of line-invariant phenotypes that are associated with patient-derived human neurons across a wide spectrum of genetic backgrounds may reveal a functional commonality that can then be investigated based on known signaling pathways and intracellular processing domains. High-throughput phenotypic analysis may also enable the identification of novel genetic interactions. By combining large-scale screening of phenotypes with high-throughput sequencing and expression analysis in neurons from genetically diverse patient cohorts, it will become feasible to identify potential synergistic effects of risk genes that individually have low penetrance across patient populations. In turn, these interactions can be modeled in a feed-forward fashion through manipulation of multiple genes in cell lines derived from disease-free control subjects or established embryonic stem cell lines. The recent development of stable intermediate neuroepithelial-like stem cells holds considerable promise for providing a source population of progenitor cells that can be easily expanded for the generation of large numbers of different neuronal subtypes amenable to genetic modification (Falk et al., 2012; Koch et al., 2009). However, for functional neuronal phenotypes that are likely to be relevant for developmental pathology, including synaptic connectivity and morphogenesis, there is still a pressing need for advanced technology that will enable efficient analysis of these cellular properties. High-throughput confocal imaging and electrophysiology are emerging platforms that will be invaluable to evaluate phenotypic features across large populations of derived human neurons.

Conclusions

Cellular reprogramming of somatic cells from patients with neurological disorders is still in its infancy, although rapid progress is being made on several fronts to optimize induction strategies, differentiation protocols, phenotypic analysis and genetic and biochemical rescue of cellular deficits. The optimal strategy for each of these parameters, however, may largely depend on the nature of the disease under investigation. Neurodevelopmental diseases may eventually require an integrated network of multiple cell types to approximate the endogeneous conditions of human brain development. For neurodegenerative diseases, identification of potential age-related catalysts in triggering cell death will be essential to gauge whether neurons derived from reprogrammed cells can effectively capture most if not all of the causal mechanisms present in endogenous conditions. All investigations of human neuronal pathology, however, will benefit from the

refinement of technology to screen large samples of human neurons for functional aberrations, whether the samples arise from a large patient cohort for polygenic disease, or a few cell lines subjected to a large library of potential bioactive compounds. Although the mechanisms of pathogenesis and effective investigative strategies may differ significantly among neurological disorders, the acceleration of technological advances created for disease-specific questions will undoubtedly benefit researchers at large interested in exploiting the potential of cellular reprogramming.

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