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Review

Disease modeling and drug screening for neurological diseases using human induced pluripotent stem cells

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With the general decline of pharmaceutical research productivity, there are concerns that many components of the drug discovery process need to be redesigned and optimized. For example, the human immortalized cell lines or animal primary cells commonly used in traditional drug screening may not faithfully recapitulate the pathological mechanisms of human diseases, leading to biases in assays, targets, or compounds that do not effectively address disease mechanisms. Recent advances in stem cell research, especially in the development of induced pluripotent stem cell (iPSC) technology, provide a new paradigm for drug screening by permitting the use of human cells with the same genetic makeup as the patients without the typical quantity constraints associated with patient primary cells. In this article, we will review the progress made to date on cellular disease models using human stem cells, with a focus on patient-specific iPSCs for neurological diseases. We will discuss the key challenges and the factors that associated with the success of using stem cell models for drug discovery through examples from monogenic diseases, diseases with various known genetic components, and complex diseases caused by a combination of genetic, environmental and other factors.

Keywords: induced pluripotent stem cells; embryonic stem cells; neurodegenerative diseases; disease modeling; drug screening

Acta Pharmacologica Sinica (2013) 34: 755–764; doi: 10.1038/aps.2013.63; published online 20 May 2013

Introduction

The causal relationships between genes and diseases are most commonly studied in animal models using gain- or loss-of-function mutations. However, in many cases, the genetic, anatomical and physiological differences between animals and humans present a challenge for projecting these relationships to human diseases. Thus far, preclinical treatments that show success in animal models have not always translated well into clinical trials, further highlighting the flaws of using the existing animal models to recapitulate human diseases^[1, 2]. Moreover, access to human tissues other than blood is scarce, and the available postmortem tissues usually only represent the late-stage characteristics of disease progression. Due to these limitations, immortalized human cell lines with genetic modifications are commonly used as cellular models for drug screening^[3]. Because of the changes introduced by immortalization, it is often questioned whether these cellular systems accurately model primary human cells.

Recent advances in stem cell research, especially the development of induced pluripotent stem cell (iPSC) technology^[4, 5], provide new opportunities which may overcome many of the challenges and shortcomings associated with disease modeling and drug screening^[6–9]. An ideal drug screening platform would provide reproducible and quantifiable disease-relevant phenotypes in a scalable cell population. iPSC-derived cells have advantages over primary cells and immortalized cell lines because they can provide inexhaustible, scalable, and genetically relevant sources for cell-based drug screening^[10, 11]. Many neurological disease phenotypes can be measured on high-content imaging platforms through changes in cellular morphology, such as soma size, neurite length, or spine density of neurons^[12]. In addition, changes in biochemical activity and gene expression could also be used as assay readouts for drug screens. Therefore, iPSCs will likely become an attractive drug discovery platform for neurological diseases.

In this review, we will discuss the progress made to date on cellular disease modeling and drug screening using stem cell systems (*i.e.*, differentiated cells from stem cells or iPSCs), with a focus on iPSCs from patients with neurological diseases. We review a few critical questions for disease modeling based on

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Received 2012-12-31 Accepted 2013-04-22

iPSCs and analyze the success and challenges of using these newly available cellular models for drug discovery.

Linking cellular phenotypes in iPSC models to genetic defects of monogenic diseases

Given that many monogenic diseases affect specific cell types with high penetrance, it is reasonable to suggest that the development of disease-relevant phenotypes is likely to be most predictable from iPSCs carrying the same genetic makeup. We will use two examples, Spinal Muscular Atrophy (SMA) and Machado-Joseph Disease (MJD), to illustrate that disease modeling with iPSCs requires connecting specific cellular phenotypes with key disease pathologies.

SMA is an early-onset neurological disease caused by the selective loss of the lower motor neurons, with symptoms including progressive muscular atrophy, paralysis and death. This disease is caused by a deletion or mutation in the survival motor neuron 1 (SMN1) gene that results in significantly reduced expression of the full-length SMN protein^[13]. The major function of SMN is to mediate the assembly of small nuclear ribonuclear proteins (snRNPs) and enable axonal mRNA transport and local mRNA processing. SMN reduction has been shown to cause both altered pre-mRNA splicing of specific target genes and reduced axonal transport of specific mRNAs to synapses, leading to the selective degeneration of the lower α-motor neurons^[14]. Moreover, some evidence has demonstrated that SMN can synergize with Bcl-2 to inhibit Fas- or Bax-mediated neuronal apoptosis^[15].

Ebert *et al*^[16] published the first study demonstrating that human iPSCs could be used to model a genetically inherited disease. This study showed that motor neurons derived from SMA iPSCs cultured for six weeks were selectively reduced in both number and size compared with those from the control groups. In addition, SMN aggregates were also reduced in the SMA iPSCs and their differentiated motor neurons. It was shown that valproic acid and tobramycin rescued the decreased number of SMN-rich structures (gems) in the SMA iPSCs. It is probable that the observed motor neuron death represented a disease-relevant phenotype that correlated with the molecular mechanism of SMN reduction. In a follow-up study, the pivotal cell death signaling mechanisms in SMA iPSC-derived motor neurons were investigated using the original patient iPSC line and a newly generated virus-free line from a second SMA patient^[17].

The activation of the Fas ligand-mediated apoptotic pathway via caspase-8 and -3 was observed in the cell death of SMA iPSC-derived motor neurons, and this cell death could be prevented by either Fas-blocking antibodies or a caspase-3 inhibitor^[17]. The causal relationship between the SMN gene defects and the selective loss of motor neurons was further demonstrated by Chang *et al*^[18], who reported that ectopic SMN expression in five SMA iPSC lines restored normal motor neuron differentiation and rescued the delayed neurite outgrowth. Therefore, they concluded that the robust phenotypes, including the reduced motor neuron production and the slower neurite development observed in motor neurons dif-

ferentiated from the multiple SMA iPSC lines, are likely due to the strong genetic determinant of the SMN1 defect.

MJD, or Spinocerebellar Ataxia Type 3 (SCA3), is a late-onset inherited neurodegenerative disease caused by the abnormal expansion of "CAG" trinucleotide repeats in the ataxin 3 (ATXN3) gene. Moreover, the size of the CAG repeat in MJD inversely correlates with the age of disease onset and directly correlates with disease severity. The ATXN3 protein is a deubiquitinating enzyme (DUB) and helps regulate the stability or activity of many cellular pathway proteins in various physiological events. The expansion of the polyglutamine (polyQ) tract in ATXN3 might alter the protein conformation, leading to changes in its interactions with native partners and to the formation of insoluble aggregates^[19]. The formation of ATXN3-containing aggregates is thought to trigger disease initiation and progression with symptoms of progressive cerebellar ataxia, although it is unknown how the aggregates elicit neurotoxicity^[20].

Recently, Koch *et al*^[21] established MJD patient-specific iPSC models and demonstrated that the L-glutamate-induced excitation of MJD iPSC-derived neurons initiated calcium-dependent proteolysis of ATXN3, followed by the formation of aberrant insoluble aggregates. Importantly, this phenotype was specifically observed in MJD iPSC-derived neurons, but not in the patient's fibroblasts, iPSCs, or differentiated glial cells. These findings could effectively explain the reason for neuron-specific degeneration in MJD, which conventional approaches have not been able to uncover. These types of neuron-dominant phenotypes demonstrate the great potential advantages of iPSC technology both in modeling monogenic neurodegenerative diseases and in studying the underlying disease mechanisms.

Other monogenic disease-specific iPSCs have been generated and display possible disease phenotypes (Table 1), including Huntington's disease (HD)^[22], Fragile X syndrome^[23], Prader-Willi Syndrome (PWS)^[24], Duchenne Muscular Dystrophy (DMD)^[25], Familial Dysautonomia (FD)^[26], and Down's syndrome (DS)^[27].

Diseases that are difficult to model using iPSCs

There are many reports that disease-specific iPSCs have been generated with little or no phenotype detected. For example, Song *et al*^[28] generated iPSCs from skin cells obtained from a relapsing-remitting multiple sclerosis (MS) patient and differentiated the MS iPSCs into mature neural lineages. However, the MS iPSC-derived neurons exhibited normal properties of functional neurons and only displayed minor differences in their electrophysiological characteristics. We speculate that the following reasons could account for the challenges of detecting disease phenotypes in iPSC models.

Late-onset diseases

Many neurodegenerative diseases are late-onset diseases, and their key phenotypes may not manifest easily within a short period of time in culture. Alzheimer's disease (AD) is a late-onset disease characterized by amyloid plaques, neurofibril-

Table 1. Selected examples of monogenic neurological diseases modeled with patient-specific iPSCs.

Disease	Genetic defects	iPSC-derived cell types	Phenotype demonstrated	Genetic rescue	Drug test	References
Spinal muscular atrophy (SMA)	Mutation in SMN1	Motor neurons	Loss of SMN1 gene expression; Reduced size and number of motor neurons.	Yes	Yes	[16–18]
Machado-Joseph disease (MJD)	Expanded CAG repeat in ATXN3 gene	Neurons and glial cells	Excitation-induced ATXN3 aggregation in differentiated neurons.	No	Yes	[94]
Familial dysautonomia (FD)	Mutation in IKBKAP	Neural crest precursors and neurons	Increased abnormal splicing in differentiated neural crest; Decreased neurogenesis and migration defects.	No	Yes	[26]
Huntington's disease (HD)	Expanded CAG repeat in HTT gene	Neural stem cells, striatal neurons, and astrocytes	Enhanced caspase3/7 activity after growth factor withdrawal; Increase in lysosomal activity in HD-iPS cells; Vacuolation in astrocytes; Increased vulnerability to stressors and BDNF withdrawal in striatal neurons.	Yes	No	[22] [95] [96] [63]

lary tangles (NFTs), and selective neuronal loss in the cerebral cortex and hippocampus^[29]. Recently, several groups reported the derivation of patient-specific iPSCs with amyloid precursor protein (APP) or Presenilin-1/2 (PS1/2) gene mutations and observed increased expression of the β-amyloid (Aβ), phospho-Tau, and activated GSK-3β^[30–32] proteins in these iPSC-derived neurons. These proteins are the critical markers in the pathological process of APP proteolysis and the formation of NFTs. However, selective cortical neuronal loss and damage to synaptic functions, which are the most important pathological changes in AD patients, were not observed. It is believed that the absence of important disease phenotypes may be a result of the immature characteristics of the neuronal culture as well as of the lack of appropriate stressors associated with ageing.

Non-cell-autonomous diseases

Selective susceptibility of neuronal cell types in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), AD, HD, and the spinal cerebellar ataxias (SCAs)^[33], can be induced by pathological changes in the neurons as well as in their interacting partners. ALS is a neurodegenerative disease that is characterized by motor neuron death in the spinal cord and brain stem^[34, 35]. In the case of the familial form of ALS, which carries the superoxide dismutase (SOD1) gene mutation, disease-specific defects will only manifest when motor neurons are co-cultured with primary cortical glial cells from SOD1G93A transgenic mice^[34] or astrocytes isolated from human fetal cerebral cortex with Lenti-SOD1^{G37R} over-expression^[35]. Although mutations in the ubiquitously expressed SOD1 gene lead to a dominant, inherited form of ALS, motor neurons derived from SOD1 iPSCs did not show any functional defects or cell loss^[36]. This observation differs from the finding that iPSCs from patients suffering from a different familial form of ALS that carries

a Tar DNA binding protein-43 (TDP-43) mutation will give rise to motor neurons with decreased survival in longitudinal studies^[37, 38]. Therefore, the disease phenotype for the non-cell-autonomous form of ALS requires proper co-culture systems. The advancement of microfluidic^[39] or 3-dimensional^[40] platforms may offer new ways to better mimic physiological, spatial, and temporal microenvironments for cell growth.

Diseases that are partly attributed to environmental stressors

Diseases that are caused by complex environmental and genetic factors may not manifest functional defects in iPSC models under basal culture conditions and might require the use of stressors to challenge the cell cultures. PD is one of the late-onset neurodegenerative disorders and is characterized by motor symptoms attributed to the death of dopamine-secreting cells in the pars compacta region of the substantia nigra in the midbrain^[41]. Recently, iPSC lines carrying the α-synuclein (SNCA) locus triplication^[42, 43] or mutations in leucine-rich repeat kinase 2 (LRRK2)^[44, 45], PTEN-induced putative kinase 1 (PINK1)^[46, 47], or parkin (PARKN)^[48] have been generated. Although PINK1 is a genetic factor for PD, it has low genetic penetrance and requires proper environmental triggers for disease onset.

Seibler *et al*^[47] generated human iPSCs from PD patients carrying either nonsense or missense mutations in the PINK1 gene, but further analysis did not reveal any defects in the generation and survival of the dopaminergic (DA) neurons differentiated from these mutant PINK1 iPSCs. However, they have observed some detectable cellular defects upon treatment with the mitochondrial stress inducer valinomycin. Therefore, it may be necessary to expose cells to the relevant biological, chemical or environmental stressors to reveal the underlying disease phenotypes when modeling late-onset, non-cell-autonomous, and complex multi-factorial diseases using iPSCs.

Progress of modeling non-monogenic neurological diseases with iPSCs

Some familial forms of neurodegenerative diseases such as PD, ALS and AD have strong genetic components, and therefore, attempts have been made to model these diseases using iPSCs, with some success (Table 2). However, diseases caused by a combination of genetic, environmental and/or other factors are difficult to model, as the epigenetic marks left by environmental and other factors are most likely erased in the process of reprogramming (Table 3). As a result, these cells may lose their ability to model complex multi-factorial disorders, especially if the epigenetic changes are critical to the manifestation of symptoms.

Of the many genetic mutations linked to PD, mutations in SNCA and LRRK2 were shown to be dominant and highly penetrant. Using PD iPSC models carrying the SNCA triplication, Byers *et al*^[42] found that the accumulation of α -synuclein and increased sensitivity to oxidative stress were specifically displayed in DA neurons derived from patient iPSCs; a similar α -synuclein pathology was also observed by another group^[43]. Nguyen *et al*^[44] found that the DA neurons derived from iPSCs carrying the G2019S mutation in the LRRK2 gene showed increased expression levels of α -synuclein and key

oxidative stress-response genes, including HSPB1, NOX1 and MAOB. Moreover, the neurons carrying the LRRK2 (G2091S) mutation were more sensitive to stress agents such as peroxide and 6-hydroxydopamine (6-OHDA). Interestingly, Sanchez-Danes *et al*^[45] found that DA neurons that were differentiated from iPSCs of either idiopathic PD or LRRK2 PD showed similar morphological alterations, including reduced numbers of neurites and neurite arborization and the accumulation of autophagic vacuoles. The genetic changes in these idiopathic PD patients captured in the corresponding iPSC lines might be sufficient to specify the functional changes in the differentiated neurons. If these results can be confirmed in future studies, it will open up possibilities for modeling sporadic diseases.

Schizophrenia (SCZD) is a complex psychiatric disorder in which genetics, early environmental factors, neurobiology, and psychological and social processes appear to be important contributors. It has been thought that a developmental disruption of neural connectivity is tightly associated with the symptoms of SCZD, which include thought disturbances, hallucinations, and decay of social and cognitive performances^[49]. Post-mortem studies revealed reduced brain volume, cell size, and spine density and abnormal neural distribution in the prefrontal cortex and hippocampus of the SCZD brain^[50].

Table 2. Selected examples of multigenic neurological diseases modeled with patient-specific iPSCs.

Disease	Genetic defects	iPSC-derived cell types	Phenotype demonstrated	Genetic rescue	Drug test	References
Amyotrophic lateral sclerosis (ALS)	Mutation in SOD1	Motor neurons	No answer	No	No	[36, 65]
	Mutation in VAPB	Motor neurons	Reduced VAPB expression in motor neurons.	No	No	[97]
	Mutation in TDP-43	Motor neurons	Formation of cytosolic TDP-43 aggregates; Decreased neurite length; Increased vulnerability to antagonism of the PI3K pathway and oxidative stressors.	No	Yes	[37, 38]
Rett syndrome (RTT)	Mutation in MECP2	Neurons	Decreased synapse number, spine number and soma size; Elevated LINE1 retrotransposon mobility.	No	No	[98–100]
	Mutation in CDKL5	Glutamatergic neurons	Aberrant dendritic spine structure.	No	No	[101]
Parkinson's disease (PD)	Mutation in LRRK2	Dopaminergic neurons and neural stem cells	Increased SNCA levels and vulnerability to stress agents; Passage-dependent deficiencies in nuclear-envelope organization, clonal expansion and neuronal differentiation.	Yes	Yes	[44–46]
	Mutation in PINK1	Dopaminergic neurons	Impaired recruitment of Parkin to mitochondria, increased mitochondrial copy number and upregulation of PGC-1 α ; Increased vulnerability to cellular stressors.	Yes	Yes	[47]
	Mutation in SNCA	Dopaminergic neurons	Increased SNCA expression and susceptibility to oxidative stress.	No	No	[42, 43]
	Mutation in PARKIN	Dopaminergic neurons	Mitochondrial dysfunction associated with increased oxidative stress and SNCA accumulation.	No	Yes	[48]
Alzheimer's disease (AD)	Mutations in PS1 and PS2	Neurons	Increased ratio of A β _{42/40} .	No	Yes	[32, 104]
	Duplication of APP	Neurons	Increased A β ₄₀ production, phospho-Tau and GSK-3 β activity.	No	Yes	[30, 31]

Table 3. Selected examples of multi-factorial neurological diseases modeled with patient-specific iPSCs.

Disease	Genetic defects	iPSC-derived cell types	Phenotype demonstrated	Genetic rescue	Drug test	References
Sporadic Parkinson's disease (sPD)	Unknown	Dopaminergic neurons	Fewer and less branched neurites, and autophagic compromise.	No answer	No	[45]
Sporadic Alzheimer's disease (sAD)	Unknown	Neurons	Higher levels of the pathological markers in one out of two patient-derived neural cells.	No answer	Yes	[30]
Schizophrenia (SCZD)	Unknown	Neurons	Decreased neuronal connectivity, fewer neurites, and decreased PSD95, glutamate receptor expression.	No answer	Yes	[51]
Multiple sclerosis (MS)	Unknown	Neurons	Neurons appear functional but have somewhat different electrophysiological characteristics than controls.	No answer	No	[28]

However, the cell types affected in SCZD and the underlying molecular mechanisms of the disease remain unclear.

Brennan *et al*^[51] directly reprogrammed fibroblasts into iPSCs from four SCZD patients who displayed either childhood onset or a familial form of the disease and subsequently differentiated these iPSCs into a largely glutamatergic neuronal population. These neurons showed diminished neuronal connectivity in conjunction with decreased neurite number, PSD95 protein levels, and glutamate receptor expression, as well as altered gene expression of components of the cyclic AMP and WNT signaling pathways. Moreover, the synaptic transmission defects of the SCZD iPSC-derived neurons were ameliorated after treatment with the antipsychotic drug loxapine for three weeks, but without the detection of synaptic functional improvement as measured by electrophysiological and spontaneous calcium imaging methods. However, four other antipsychotic drugs failed to improve the neuronal connectivity, raising questions about the significance of the protective effects of loxapine in these iPSC models.

The key enabling factors in cellular disease models using iPSCs

We propose here that the following three critical factors need to be considered when using iPSCs for disease modeling and compound screens.

Efficient differentiation of iPSCs into cell types impacted by diseases

Recently, specific functional neural cells have been successfully obtained from iPSCs, including neural progenitor cells (NPCs), DA neurons, cholinergic neurons, motor neurons, astrocytes and oligodendrocytes^[52-55]. However, the current differentiation methods, which are based on developmental paradigm or relevant cell fate regulation signals, require time-consuming, laborious and complicated processes and often result in a low percentage of the desired cells with immature characteristics. Therefore, it is critical for investigators to establish robust and efficient differentiation protocols for obtaining fully differentiated disease-relevant cells. Fortunately, the generation of transgenic reporter cell lines using fluorescent reporter genes under the control of cell-type-specific promoters can help to

track and purify disease-relevant cells from heterogeneous differentiated cell types. For example, the HB9:GFP human embryonic stem cell (ESC) reporter line has been used to generate motor neurons for studying ALS^[56, 57]. The advances in genetic engineering methods such as the use of zinc finger nuclelease (ZFN)^[58] and transcription activator-like effector nucleases (TALEN)^[59] offer great tools for lineage-specific labeling in iPSCs.

Detection of genotype-associated disease phenotypes

Building solid links between genetic mutations and disease-specific phenotypes in iPSCs would provide confidence for drug discovery using these models. For neurological diseases, disease-related phenotypes can be observed via deficits in cell survival, neuronal morphology, neuronal migration/neurogenesis, synaptic function, and electrophysiological function. Biochemical changes that are directly linked to gene mutations are easily detectable. For example, reduced SMN1 protein expression was found in SMA iPSCs^[16], and α -synuclein accumulation was observed in DA neurons differentiated from iPSCs carrying either the SNCA triplication or the LRRK2 mutation^[42, 44]. Cellular functional changes that are linked to disease symptoms are not easy to establish, yet they are more valuable for compound screens.

HD is a dominantly inherited late-onset neurodegenerative disease caused by a CAG repeat expansion in the first exon of the gene huntingtin (HTT)^[60], and it is characterized by the selective loss of medium spiny projection neurons in the striatum and cortex of the HD brain. It is generally accepted that mutant HTT with greater than 36-38 CAG repeats may mediate neurodegeneration via the generation of toxic N-terminal fragments, alterations in vesicular trafficking, mitochondrial function and transcriptional dysregulation^[61, 62].

The HD Consortium^[63] generated and characterized a panel of 14 iPSC lines derived from controls and HD patients with varying numbers of CAG repeats and timeframes for the age-onset of the disease. Microarray profiling revealed that these iPSC lines have clear, reproducible CAG repeat-expansion-associated phenotypes upon differentiation, and distinctly different gene expression profiles were found between patient iPSC lines and those derived from controls. Differences in

the gene expression profiles were also found between early-onset HD iPSCs and late-onset iPSCs. Disease-associated changes in electrophysiology, metabolism, cell adhesion, and ultimately cell death were shown in the differentiated neural cells from HD iPSCs lines harboring both medium and longer CAG repeat expansions. Cells carrying the longer repeats were more vulnerable to cellular stressors and BDNF withdrawal when assessed using a range of assays across consortium laboratories^[63], supporting the correlation of CAG repeat lengths with disease onset.

Confirmation of disease phenotypes with genetic rescue experiments

The most powerful proof of disease phenotypes in iPSC models comes from the genetic rescue of the phenotypes by correcting gene mutations, as illustrated in the case of SMA that was discussed in an earlier section^[18]. Another example was given by An *et al*^[64], who reported that HD iPSCs were corrected through the replacement of the expanded CAG repeat with a normal repeat using homologous recombination and demonstrated the restoration of function in differentiated DARPP-32(+) neurons both *in vitro* and *in vivo*. Moreover, the genetic correction of HD iPSCs normalized the pathogenic HD signaling pathways and reversed the relevant disease phenotypes such as the susceptibility to cell death and the altered mitochondrial bioenergetics in neural stem cells^[64]. DA neurons differentiated from PINK1 iPSCs^[46, 47] displayed impaired mitochondrial function, as shown by the disabled stress-induced mitochondrial translocation of parkin, increased mitochondrial copy number and upregulation of PGC-1α. Importantly, these phenotypes were rescued by the lentiviral expression of wild-type PINK1 in neurons derived from PINK1 iPSCs^[47]. Rescue experiments can therefore provide definitive proof that the phenotypes observed in the iPSC models are indeed due to the specific genetic defects.

The iPSC technology-related challenges for disease modeling

As many labs are generating disease-specific iPSCs, clone variations have been observed to affect the differentiation potential and phenotypes of iPSCs. For example, Boultling *et al*^[65] generated 16 iPSC lines from seven different individuals of varying age, sex and health status. After characterization, three of the iPSC lines were found to be resistant to neuronal differentiation. In this section, we will discuss the main factors causing phenotype variations among iPSC clones and suggest possible solutions for them.

Genetic aberrations

Currently, most iPSCs are generated using reprogramming factors transduced by integrating viral vectors such as lentivirus or retrovirus, which often cause mutations at the integration sites or other genetic aberrations such as copy number variations or abnormal karyotypes^[66]. Genetic alteration by random viral integration may affect the differentiation of iPSCs as well as their phenotypes. For example, Somma *et*

al^[67] found that the removal of the reprogramming transgenes improved the developmental potential of iPSCs and augmented their capacity to undergo directed differentiation *in vitro*.

Strategies have been developed for the generation of transgene-free iPSCs to minimize or eliminate genetic variations. Non-integrative approaches using excisable lentiviral or transposon vectors^[68], non-integrating RNA viruses or Sendai viruses^[69], episomal vectors^[70], mRNA transfections^[71], and recombinant proteins^[72] have been developed for reprogramming. In addition, a series of small molecules such as 5-aza-dc, vitamin C, valproic acid and forskolin have been reported to improve iPSC reprogramming efficiencies^[73]. Successful examples of integration-free patient iPSCs include those from SCZD patients harboring a DISC1 mutation^[74] and idiopathic PD patients^[75].

Epigenetic memory in iPSCs

Several groups have shown that iPSCs retain epigenetic memory from their donor cells^[76-79]. Lister *et al*^[77] discovered that iPSCs displayed significant reprogramming variability, including somatic memory and aberrant reprogramming of DNA methylation, which were independent of the reprogramming techniques. This type of epigenetic memory would influence the differentiation potential of iPSCs. For example, Bar-Nur *et al*^[76] reported that β-cell-derived iPSCs displayed an increased ability to differentiate into insulin-producing cells compared with ESCs and isogenic non-β cell-derived iPSCs. Some studies have indicated that long-term culture of iPSCs with increased passage number may decrease the differences between iPSCs and ESCs, followed by the loss of parental cell line characteristics^[78].

The absence of well-defined controls

Currently, iPSCs from age-matched, unaffected donors are usually chosen as controls in iPSC disease models. However, these controls are not ideal for iPSC disease models as they usually have different genetic backgrounds and a different history of risk factor exposure. The use of gene editing technologies such as ZFN and TALEN to correct disease genes in iPSCs might be helpful to generate lines which can serve as isogenic controls^[80]. In addition, temporal changes in differentiated cells from disease or control iPSCs can reveal subtle phenotypes in a very sensitive way if compared with the baselines of each cell type. For example, selective motor neuron death occurred in 6-week differentiated SMA neurons, but not in 4-week differentiated ones^[16]. Moreover, a recent study that established iPSC lines from centenarians^[81] may provide valid controls for studying late-onset diseases using iPSC models, as the donors were presumed to have no serious disease risk factors.

Lack of large numbers of iPSC lines

As each individual patient has a distinct genetic background and many pathways contribute to the cellular phenotypes, it is often difficult to pinpoint a cellular phenotype to a specific

gene mutation. This can be further complicated by the various co-morbidities in individuals. The key disease-relevant phenotypes should be shared by iPSCs bearing the same gene mutations even though the cells were derived from patients with different genetic backgrounds. The discovery of common cellular phenotypes from a large panel of patient iPSCs could increase the reliability of the cellular defects associated with the gene mutations carried by the iPSCs. Drug screening based on these disease phenotypes beyond clone and donor variations would be reliable and ideal for discovering new drugs. As an example, the HD consortium obtained a total of 14 iPSC lines and demonstrated consistent phenotypes, with severity correlating with the extent of the genetic mutations (*ie*, CAG repeat expansion)^[63].

Therefore, it is important to use more than one iPSC line from each patient and control, and select a diverse group of patients with different genetic backgrounds to ensure that disease-specific cellular phenotypes are detected beyond the normal variability of iPSC-derived cells through an appropriate number of iPSC lines.

Other stem cell-based approaches for disease modeling beyond patient-specific iPSCs

In addition to the exciting progress made in patient-specific iPSCs for disease modeling, a few other technical breakthroughs can likely offer convenience, broaden patient scope, and even tighten disease relevance. We will review some of these approaches here.

Disease modeling by challenging cells derived from normal iPSCs or ESCs

Toxin challenge and the overexpression of disease genes have been widely used in cell lines or primary rodent cells for disease modeling. True human cells can now be used for these experiments due to the availability of iPSCs and ESCs, which might represent a major improvement in such established approaches. Xu *et al*^[82] modeled neuronal loss in human AD by challenging forebrain neurons differentiated from human iPSCs with A β_{1-42} aggregates. Several small molecules were identified as potent inhibitors of the cellular toxicity elicited by the A β aggregates via the screening of a few compound sets^[82]. Another approach is the generation of a panel of isogenic cell lines by expressing disease-causing transgenes in wild-type ESCs or iPSCs. For example, Soldner *et al*^[83] have established the A53T (G209A) α -synuclein mutation in human ESCs using ZFN technologies.

Disease modeling using desired cells differentiated from patient adult stem cells

One of the most exciting advances in disease modeling comes from isolating adult stem cells directly from the postmortem tissues of patients. Haidet-Phillips *et al*^[84] isolated adult NPCs from the lumbar spinal cord tissue of ALS patients, and the astrocytes derived from those NPCs were co-cultured with lineage-labeled motor neurons from non-ALS-affected ESCs. The astrocytes derived from the NPCs of familial ALS patients

carrying the SOD1 mutation demonstrated significant toxic effects to motor neurons. Unexpectedly, astrocytes derived from the NPCs of sporadic ALS patients also demonstrated toxicity to motor neurons, which offered a novel cell system to screen compounds with a broader scope of cell defects beyond the previous known gene mutations. As the majority of diseases are not monogenic diseases or diseases with known genetic components, the approaches demonstrated here could have a great impact on disease mechanism studies as well as compound screens if widely adopted.

Disease modeling using iNPCs and iNs directly generated from patient somatic cells

Generating neurons or NPCs from ESCs and iPSCs is a complicated and time-consuming process that could introduce batch-to-batch variations and increase the risk of neoplasia or undesired cell types. This process can be circumvented by the direct conversion of somatic cells from one cell type to another via the ectopic expression of specifically defined transcription factors. Recently, cell types ranging from NPCs^[85, 86] to neurons^[87, 88], including motor neurons^[89] and DA neurons^[90], were able to be derived from human fibroblasts. These NPCs and neurons were named iNPCs and iNs, respectively. For example, Tian *et al*^[86] found that five different transcription factors could directly convert adult dermal fibroblasts into iNPCs, and the resulting cells possessed similar properties to the primary NPCs, including proliferation, self-renewal, differentiation, and chemotactic properties. However, this type of strategy is not sufficient to provide cells at a large enough scale for some applications, including drug screens. Additionally, this approach has the disadvantage of potentially skipping the neuronal developmental stages in which some disease phenotypes may become evident. Whether this type of technology could provide an alternative strategy for disease modeling and drug screening remains to be seen.

Conclusions and perspectives

With an ever-growing list of patient-specific iPSCs available, many attempts to use iPSCs for disease modeling and drug screening have been explored. Success in this area requires the development and characterization of reliable disease phenotypes from both iPSCs and their derivatives. Advances in iPSC technologies have reduced the phenotypic variations among iPSC clones, enabling the establishment of reliable disease models for drug screening. In addition, some alternative stem cell-based approaches such as deriving iNPCs/iNs directly from somatic cells or NPCs derived from postmortem tissues might help to establish novel neurological disease models for drug discovery. Using panels of iPSCs from broad patient populations, compounds could potentially be profiled in these cells, representing “clinical trials in test tubes”^[91]. This could be a potential method by which iPSCs contribute to personalized medicine, drug testing, and predictive toxicology studies^[92]. Testing drug candidates in iPSCs carrying distinct genetic mutations of certain diseases might help to choose the appropriate patient populations for the lead molecules. Effec-

tive patient stratification is expected to reduce clinical trial cost and also attrition rates^[93]. Therefore, it is anticipated that disease modeling and compound screens on iPSC models will lead to important breakthroughs in drug discovery and development.

Acknowledgements

The authors would like to thank Jing ZHAO, Hai-yan FANG (both from GlaxoSmithKline R&D, China), Dwight MORROW (GlaxoSmithKline, USA), Kevin J KIM (Harvard Stem Cell Institute, USA) and Lucas CHASE (Cellular Dynamics International Inc, USA) for their critical reading of the manuscript and valuable suggestions.

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