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EXPERT REVIEW

Cellular Reprogramming: A New Technology Frontier in Pharmaceutical Research

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ABSTRACT Induced pluripotent stem cells via cellular reprogramming are now finding multiple applications in the pharmaceutical research and drug development pipeline. In the pre-clinical stages, they serve as model systems for basic research on specific diseases and then as key experimental tools for testing and developing therapeutics. Here we examine the current state of cellular reprogramming technology, with a special emphasis on approaches that recapitulate previously intractable human diseases *in vitro*. We discuss the technical and operational challenges that must be tackled as reprogrammed cells become incorporated into routine pharmaceutical research and drug discovery.

KEY WORDS cellular reprogramming · drug discovery · iPSC · pharmaceutical research · stem cells

INTRODUCTION

The availability of physiologically relevant human disease models could streamline preclinical testing and reduce costly drug failures in later stages of clinical testing. Studies of human disease rely heavily on transgenic animal models for elucidation of the molecular mechanisms that underlie pathological states. Despite their utility, murine disease models are not available for all diseases and in some cases, existing models fail to faithfully recapitulate key aspects of the human disease phenotype. This can be attributed to fundamental biological and genetic discrepancies between the two species, impeding proper drug intervention and suitable targeted therapies. For instance, although human cardiovascular biology is typically

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studied in mouse systems, the murine heart rate is ten times faster than that of humans, making it challenging to study human cardiac arrhythmia or other electrophysiological disorders . Both species exhibit a similar form of double outlet right ventricle (DORV) congenital heart disease, yet survival varies significantly (1). Other examples of species-specific physiology and phenotypic variation due to differences in gene-dosage sensitivity can also be seen in studies of Fanconi anemia (2), Down's syndrome (3,4) and other congenital heart diseases (1). Furthermore, human clinical studies require large quantities of patient- and/or disease-specific tissues and cells (such as liver, pancreas, heart, neurons), which are generally difficult to obtain. Immortalized cell lines serve as a convenient source of biomaterial but introduce additional confounding factors in that prolonged growth in cell culture may give rise to karyotypic abnormalities, phenotypic variations and clinically irrelevant responses to drug compounds (5).

Human pluripotent stem cells, characterized by their ability to self-renew and to generate all somatic cells and tissues in the body, offer a powerful alternative approach to disease modeling. They can be used to study early developmental processes, and to identify the effects of specific mutations on various cell types, which are otherwise inaccessible for research. Over the past few years, groundbreaking research conducted by Takahashi and Yamanaka shed light on the stem cell field, when it was shown that the induced expression of few defined transcription factors enabled the reprogramming of somatic cells into human induced pluripotent stem cells (hiPSCs) (6,7). hiPSCs are similar to human embryonic stem cells (hESCs) in many ways, and given their ability to generate cells comprising of the three germ layers *in vitro* and *in vivo*, and their unlimited self-renewal capacity, these human pluripotent stem cells have the potential to represent the much needed disease models, and reflect human pathophysiology.

Recent advances have demonstrated the utility of hiPSCs reprogrammed from easily accessible, abundant sources of patient-derived tissue to model specific diseases "in a dish" (8–10); ideally these cells capture the disease phenotype when differentiated into the relevant disease cell type. These patient-specific hiPSCs and their differentiated derivatives contain the genetic background of the patient, allowing researchers to identify critical genotype-phenotype relationships in both monogenic and complex diseases (5). This is particularly crucial in disease modeling and subsequent drug screening efforts, since different subsets of patients with the same genetic condition may present varying disease severity and phenotype penetrance, possibly due to the interaction of modifier genes with the disease loci (11). Patient-specific, hiPSCs-derived cells are also immunologically matched to their respective patient to potentially prevent rejection following transplantation in cell replacement therapies. Furthermore, these unlimited supplies of cells facilitate novel biological studies, including drug discovery and toxicology testing, which would be difficult using animal models or primary human cell cultures (12).

In this review, we focus on to the strategies for cellular reprogramming by hiPSCs generation as well as dedifferentiation and transdifferentiation, and discuss their applicability in disease modeling, cell-based therapies and drug development (Fig. 1). We further highlight the present status and prospects for hiPSCs application in the development of new disease models, the drug discovery process, and personalized and regenerative medicine. Although the appropriate use of hiPSCs as transplantation therapies is likely to take more years, they have enormous potential to improve disease models and to better understand the disease mechanism in the near term. In these incipient stages of hiPSCs-based disease modeling, we examine the limitations of current reprogramming and *in vitro* directed differentiation protocols and identify the issues that must be addressed before hiPSCs-derived cells can become a routine component of mechanistic studies, drug-screening assays and various pharmaceutical researches.

Cellular Reprogramming

Before the term "reprogramming" was known, hESCs were regarded as the gold standard for cell replacement therapy and disease modeling. These pluripotent stem cells were first derived in 1998 by Thomson and colleagues from inner cell masses (ICM) of human blastocysts (13). Mechanistic studies were henceforth performed in hESCs to elucidate disease mechanisms; for instance, disease-specific hESCs can be isolated either from embryos subjected to preimplantation genetic diagnosis (PGD), or by *in vitro* mutagenesis of known genetic loci associated with a disease trait (14,15). Although hESCs have been generally useful in dissecting disease mechanisms (as demonstrated in Lesch-Nyhan-specific hESC lines, which successfully recapitulated the disease phenotype of increased uric acid production as a result of hypoxanthine–guanine phosphoribosyltransferase (HPRT) mutations, allowing further insights into the disease mechanisms and enabling drug screening(16)), their use in ESC-based therapy has resulted in moral and ethical issues associated with the requisite blastocyst destruction. In addition, clinical studies with hESCs are limiting as PGD is restricted to the diagnosis of embryos coming from a limited group of genetic disorders such as Fragile X

syndrome (17), cystic fibrosis (18), and sickle cell disease (19).

The successful reprogramming of human fibroblasts to pluripotent stem cells (hiPSCs) by retroviral transduction of four transcription factors (Oct4, Sox2, Klf4 and Myc: OSKM) was achieved in 2006 by Shinya Yamanaka and colleagues (7), based on their earlier research performed on mouse embryonic fibroblasts (MEFs) (6). This dogma-shattering discovery changed global perspectives in cell and developmental biology, and had profound implications in the development of human disease models, as well as drug discovery (20). Since that time, hiPSCs lines have been reprogrammed from numerous other human cells and patient-specific samples (21–23).

Reprogramming of somatic cells to hiPSCs depends on the inherent cellular plasticity of the starting cell population. Viral-mediated reprogramming with integrating retro-viruses or lenti-viruses remains a general strategy to generate patient-specific hiPSCs for disease modeling, resulting in genetic modification of the reprogrammed cells. However, this greatly compromises the downstream utility of hiPSCs in cell-based therapies, as the threat of insertional mutagenesis of the host gene and/or reactivation of the oncogene Myc remains a concern (14). Moreover, the residual virus vectors may influence gene expression, biological properties and/or the observed phenotype of the hiPSCs differentiated derivatives, which additionally could make it difficult to observe potential low-penetrance phenotypes in complex diseases (5,14).

In light of these concerns, several other reprogramming approaches have been developed including, but not limited to: non-integrating strategies using adenoviral (24), Sendai and episomal vectors (25), synthetic modified mRNA reprogramming (26), use of small molecules(27), and microRNA (miRNA) reprogramming (28). Although the safety issue of random viral integration is eliminated in the non-integrating strategies, they are dramatically slower and less efficient. There exists the possibility of vector subfragment integration into the hiPSCs, which could still interfere with basal gene expression (14). A recent paper describes the transfection of synthetic modified mRNAs encoding the four Yamanaka factors for RNA-mediated reprogramming of differentiated cells; this highly efficient method, combined with the lack of genetic modification holds promises for various reprogramming applications (26). Recently, it was shown that miRNA-mediated reprogramming presents a novel and highly efficient method for generating mouse and human iPSCs, without employing the Yamanaka factors; the efficiency was reported to be of two orders of magnitude higher than that of Yamanaka factor expression(29). The authors further demonstrated that miR367 expression and Hdac2 suppression are both required for miR302/367-mediated reprogramming, to activate Oct4 expression (29). This strategy may present a cornerstone technology for future reprogramming applications, and allow further adaption for rapid generation of patient-specific hiPSCs in clinical studies.

Taking this idea one step further, recent studies have demonstrated that small molecules may take the place of transcription factors and may increase the speed and efficiency of reprogramming so as to reduce the risk of undesirable characteristics acquired in culture (20). For example, Klf4 and Myc may be replaced by valproic acid, a histone deacetylase inhibitor, to reprogram neonatal human fibroblasts (30). The compound E-616452 (also known as RepSox) can take substitute for Sox2 in MEF reprogramming, by inhibiting the transforming growth factor-beta (TGF-β) receptor to allow upregulation of Nanog expression (31). Kenpaullone, a GSK3 inhibitor, has been shown to effectively replace Klf4 during reprogramming of MEFs overexpressing Oct4, Sox2 and Myc (32). A combinatorial treatment with small molecules Alk5 inhibitor SB431542, MEK PD0325901 and thiazovivin increased reprogramming efficiency greater than 200-fold in contrast to the four-factor reprogramming (33). The reprogramming of somatic cells to hiPSCs via small molecules alone has yet to be demonstrated and if feasible, would open up enormous opportunities for the field in drug discovery.

Dedifferentiation

Studying the mechanisms of inherent tissue regeneration has produced key insights in the field of regenerative medicine. Dedifferentiation refers to the reversion of terminally differentiated cells into a previously less differentiated cellular state, allowing the latter to proliferate. This process potentially replaces tissue loss arising from disease, aging and/or injury, and the dedifferentiated cells are redifferentiated into the target cell type. This regenerative phenomenon, though limited in humans, is generally observed in other non-mammalian vertebrae. For instance, regeneration of the heart is possible in zebrafish, even after amputation of up to 20% of the ventricle (34). Urodele amphibians exhibit extensive regenerative capacity after limb amputation; cells adjacent to the wound dedifferentiate, proliferate and finally redifferentiate to regenerate all of the component tissues of the limb (35). In mammals, following nerve injury, Schwann cells dedifferentiate and later proliferate (36).

Although inherent regenerative capacity is lacking in humans, in some cases experimentally induced dedifferen-

tiation can regenerate tissues; for instance, investigators have been trying to dissect the mechanisms by which cardiomyocytes dedifferentiate and then proliferate. Evidence suggests that the dedifferentiation of mammalian cardiomyocytes can be experimentally induced by both FGF1 stimulation and p38 MAPK inhibition, followed by disassembly of the contractile apparatus, before they proliferate (37). However, treatment of the cells with neuregulin showed that dedifferentiation might not be necessary for the cardiomyocytes to proliferate (38). Current protocols are still limited in understanding the regenerative phenomenon, to be readily applied in disease modeling approaches, and therefore require further optimization and inquiry from investigators.

Transdifferentiation

Ideally, a faster method of obtaining desired cell populations from patient-specific biomaterial would bypass the pluripotent intermediate, thus ruling out the need to optimize the requisite reprogramming protocol before performing the crucial *in vitro* directed differentiation step. Transdifferentiation describes the interconversion of one fully differentiated cell type directly into another without first passing through a multipotent or pluripotent intermediate (39). This has opened new avenues for the rapid generation of therapeutically desirable and immunologically-compatible cell types such as cardiomyocytes, hepatocytes and neurons from readily available patient-derived cells.

Historically, it was believed that successful transdifferentiation would be possible only if the starting and desired cell types come from the same germ layer and/or lineage(40). However, several more recent studies suggest otherwise, and demonstrate that transdifferentiation can indeed proceed across germ layers. In 1987, it was first demonstrated that mouse fibroblasts can be directly converted into myoblasts by overexpressing MyoD (41). Similarly, postnatal cardiac or dermal fibroblasts can be transdifferentiated into cardiomyocytes with the use of three developmental transcription factors Gata4, Mef2c and Tbx5 (42). In another scenario, the direct conversion of mouse fibroblasts to functional neurons, termed 'induced neurons' (iNs) was demonstrated by Vierbuchen and colleagues in 2010 by ectopic factor expression (43); these findings were further translated to human fetal and postnatal fibroblasts for the transdifferentiation of human iNs (hiNs) in 2011 (44). Nevertheless, these hiNs require co-culturing with murine cortical neurons in order to form functional synapses. The direct transdifferentiation of human postnatal and adult primary dermal fibroblasts into functional neurons has been demonstrated using a combinatorial treatment of miR-124 and transcription factor MYT1L and BRN2 under chemically defined conditions (45). These hiNs displayed characteristic neuronal morphology, marker gene expression and electrophysiological profiles. Additionally, they form functional synapses when cultured alone, without a second cell type. This study represents a major advance in reprogramming technology, accomplishing rapid and efficient conversion of adult cells into therapeutically desirable neuronal cells. Other examples of transdifferentiation include the reprogramming of pancreatic acinar cells to insulin-secreting β-cells with Ngn3/Neurog3, Pdx1 and Mafa (46), and transcription factor CCAAT-enhancer-binding protein-α-induced transdifferentiation of B cells to macrophages (47).

Collectively, these studies represent a general paradigm of reprogramming for the rapid production of *in vitro* human disease models from patient-specific cells. Despite the impressive progress made to develop strategies designed to control cell fate, skeptics can argue whether these hiPSCs-derived cells display similar disease phenotypes as those of the patient, or whether these cells can functionally replace the diseased cell type *in vivo*. Furthermore, successful *in vitro* modeling of complex diseases with multicellular phenotypes using hiPSCs or hESCs has yet to be demonstrated. Nevertheless, monogenic diseases will serve as proof-of-principle models for developing reliable differentiation and phenotyping assays, and for future investigation of polygenic conditions.

IPSCS IN NOVEL DISEASE MODELS

Recent advances in hiPSCs research have significantly changed our perspectives on personalized and regenerative medicine by providing researchers with a unique tool to derive disease-specific stem cells for study. Generating *in vitro* disease models using hiPSCs technology will offer a simplified dynamic model, not only for elucidating mechanisms of disease but also for drug screening. hiPSCs-based disease models have been successfully generated from patients with various diseases or disorders (Table I).

Models of Cardiovascular Diseases

Several groups have generated hiPSCs lines from patients with cardiovascular disease, and these have recapitulated the disease phenotype in culture. One disease model focuses on the ion channel mutation in patients with Long QT Type 1 syndrome (LQT1) (48–50). A mutation in the KCNQ1 (potassium voltage-gated channel, KQT-like subfamily, member 1) gene involved in generating cardiac action potentials results in an elongated QT-interval. hiPSCs lines were generated from two related patients from a family with an R190Q missense mutation in

Table I List of Human iPSC Disease Models

Disease type	Disease name	Genetic etiology	Cell type	Disease phenotype	Drug test	Reference
Cardiovascular Disease	Long QT 1 syndrome	Monogenic; missense mutations in the KCNQ1 gene	Cardiomyocytes	Increased cardiomyocyte depolarization	Yes;Increased susceptibility to catecholamine -induced tachyarrhy -thmia and that betablockade attenuated this phenotype.	(48)
	Long QT 2 syndrome	Monogenic; A614V missense mutation in the KCNH2 gene	Cardiomyocytes	Increased cardiomyocyte depolarization	Yes; Pinacidil completely abolished all EADs and triggered arrhythmias in all LQTS iPSC-CMs; ranolazine did not significantly alter APD90 or cFPD in LQTS cardiomyocytes	(49)
	Timothy syndrome	Monogenic; a single amino acid substitution in exon 8a of CACNA1C	Cardiomyocytes	Increased cardiomyocyte depolarization	Yes; Roscovitine increases the voltage-dependent inactivation of Ca(V)1.2, restored the electrical and Ca(2+) signalling properties of cardiomyocytes from Timothy syndrome patients.	(50)
	Hutchinson-Gilford progeria syndrome (HGPS)	Monogenic; a truncated and farnesylated form of Lamin A	Smooth muscle cells	Absence of progerin,lack of the nuclear envelope, epigenetic alterations, and appearance of premature senescence phenotypes.	N.D.	(105,106)
	LEOPARD syndrome	Monogenic; a mutation in the PTPN11 gene	Cardiomyocytes	Increased cardiomyocyte size, decreased MAPK signaling.	N.D.	(51)
Neurological Disease	Parkinson's Disease (PD)	Polygenic	Dopaminergic neurons	No obvious defect	N.D.	(107)
	Parkinson's Disease (PD)	Polygenic LRRK2 mutation	Dopaminergic neurons	Neuronal death with chemicals	Yes; Hydrogen peroxide, MG-132, and 6-hydroxydopamine increased the sensitivity of differentiated cell derived from iPSCs to caspase-3 activation and cell death	(108)
	Amyotrophic lateral sclerosis (ALS)	Polygenic	Motor neurons	N.D.	N.D.	(56)
	Spinal muscular atrophy (SMA)	Monogenic; mutations in the survival motor neuron 1 gene (SMN1)	Motor neurons	Reduced size and number of motor neurons, loss of SMN gene expression, defective synapse formation	Yes; VPA and tobramycin increased total SMN1 protein andgem formation	(54)
	Familial dysautonomia (FD)	Monogenic; mutation in the IKBKAP gene	Neural crest cells	Express low levels of KBKAP; marked defects in neurogenic differentiation and migration behaviour	Yes; Kinetin treatment markedly decrease in mutant IKBKAP form, but it did not result in a significant increase in the expression of neurogenic markers or improve migration Behaviour; continuous kinetin treatment induced a significant increase in the percentage	(61)

Table 1 (continued)

Disease type	Disease name	Genetic etiology	Cell type	Disease phenotype	Drug test	Reference
					of differentiat -ing neurons and in the expression of key peripheral neuron markers.	
	Rett syndrome	Monogenic	Neurons	Loss of synapses, reduced spine density, smaller soma size	Yes; IGF1, high dose gentamicin treatment led to more glutamatergic synapses; decreased frequency/intensity of spontaneous currents	(53)
Hepatic Disease	A1-antitrypsin deficiency (A1ATD); glycogen storage disease type 1a (GSD1a)	Monogenic; homozygous Glu342Lys; Absent hepatic glucose-6 -phosphatase enzyme	Hepatocytes	Aggregation of misfolded alpha1-antitrypsin in the endoplasmic reticulum	Yes; glucagon stimulation can induce the expression of 3 canonical glucagons -responsive genes in cells generated from GSD1a-iPSCs	(109)
	Familial hypercholesterolemia (FH)	Monogenic; Autosomal dominant mutation in LDL receptor	Hepatocytes	Deficient LDL receptor-mediated cholesterol uptake; elevated lipid and glycogen accumulation	N.D.	(109)
Pancreatic Disease	Type 1 diabetes	Polygenic	Insulin and glucagon producing cells	N.D.	N.D.	(57)
Others	Prader-Willi syndrome (PWS)	Monogenic; partial translocation of the paternally expressed chromosome 15q11-q13 region to chromosome 4.	Neurons	Reduced expression of the disease associated small nucleolus RNA HBII-85/ SNORD116	N.D.	(110)
	Angelman and Prader-Willi syndrome	Monogenic; loss of function of UBE3A gene	Neurons	Loss of paternal UBE3A expression	N.D.	(111)

KCNQ1. Cardiomyocytes differentiated from hiPSCs from these patients exhibited prolonged action potentials in single-cell electrophysiological assays. LQT1 can also be triggered by certain drugs in sensitive individuals. With patient-specific functional (beating) cardiomyocytes from sensitive individuals it is now possible to test drugs *in vitro* for the possibility that they may cause this side effect in sensitive individuals.

Another work by Lemischka *et al.* produced cardiomyocytes from two hiPSCs lines derived from two patients 25-year-old female and a 34-year-old male,with a heterozygous T468M substitution in PTPN11 which leads to LEOPARD syndrome (51). LEOPARD syndrome affects various parts of the body; symptoms generally include hypertrophic cardiomyopathy (HCM), facial dysmorphisms, lentigines or liver spots, growth retardation, abnormal genitalia and deafness. The authors found that hiPSCs-derived cardiomyocytes of patients with LEOPARD syndrome were enlarged, possibly reflecting the hypertrophic cardiomyopathy associated with this disease. Furthermore, the cell model reproduced defects in key signaling pathways and molecular targets related to the disease. In contrast to control cell lines, iPSCs-derived cardiomyocytes of patients with LEOPARD displayed increased EGFR and MEK1 phosphorylation, and failed to phosphorylate ERK (pERK) in response to basic fibroblast growth factor (bFGF) stimulation. This study illustrates the valuable utility of patient-specific hiPSCs to elucidate disease mechanisms and pinpoint affected signaling pathways.

Models of Neurological and Neurodegenerative Diseases

Neurological and neurodegenerative diseases are another class of disorders that have been successfully modeled. In 2009, Hotta *et al.* first derived a hiPSCs line from an 8-year-old, Rett syndrome (RTT) patient possessing the heterozygous R306C missense mutation in MECP2 (52). In 2010, Marchetto *et al.* successfully developed a culture system using hiPSCs from RTT patients' fibroblasts (53). This iPSCs model was used to examine autism in the lab and study RTT molecular pathogenesis. RTT-specific hiPSCs typically give rise to glutaminergic neurons with fewer synapses and decreased calcium transients. These findings revealed disease-specific molecular and functional defects, and demonstrated that these symptoms are reversible, suggesting new avenues for the identification of druggable targets in autism treatment.

Spinal muscular atrophy (SMA) is an autosomal recessive, neurogenetic disorder caused by SMN1 gene mutation, which resulted in reduced expression of SMN1 and progressive loss of lower motor neurons. Ebert *et al.* generated hiPSCs from patients with Type I SMA containing partial deletions of SMN1 gene (54). *In vitro* survival studies indicated that motor neurons derived from the SMA patient-specific hiPSCs were initially similar in morphology and number, compared to those derived from wild-type hiPSCs. Upon *in vitro* differentiation, a significant decrease was observed in motor neurons size and number, but not the total neuron pool, which may reflect the developmental loss of motor neurons observed in disease progression. Importantly, this deficiency in SMN levels could possibly be reversed with valproic acid or tobramycin treatment, thus providing a basis for future drug screens (55).

Dimos *et al.* have generated hiPSCs from a patient with the familial form of amyotrophic lateral sclerosis (ALS) and differentiated motor neurons produced from these hiPSCs, providing an unprecedented *in vitro* resource for elucidating the molecular mechanism of motor neuron death in ALS patients (56).

The Advantage of *In Vitro* Disease Models Derived from hiPSCs

The most significant advantage of disease models derived from hiPSCs is that they offer the promise of studying human cells, which may reflect a disease phenotype more accurately than currently used *in vitro* or animal models. This aspect promotes more efficient development of therapeutics and potentially, the allows for the possibility of customized medicine for individual patients. Type 1 diabetes (T1D) (57), which has unclear genetic and molecular etiology, is marked by autoimmune destruction of insulin-secreting pancreatic beta cells. The study of T1D has been impeded by the lack of a predictive disease model that can be experimentally manipulated *in vitro*. The initiation of the primary disease process often occurs long before the patient shows any sign of disease, thus, the relevant tissues can be limited and difficult to obtain. Although insight into the pathogenesis of T1D comes largely from rodent models such as the non-obese diabetic (NOD) mouse or the BioBreeding (BB) rat, these rarely fully recapitulate the human disease (58–60). Indeed, insights from rodent models have frequently not translated well to the clinic. T1D-specific iPS (DiPS) cells derived from patients offer several significant advantages. Firstly, DiPS cells would unquestionably contain the genotype responsible for T1D. Secondly, DiPS cells would provide an immunologically matched autologous cell population, although dependent on improvements in differentiation protocols. Thirdly, patient-specific cells make possible patient-specific disease modeling wherein the initiation and progression of this poorly understood disease can be studied. Because DiPS cells can be manipulated and studied *in vitro*, one could envision the

generation of specific cell types of the immune system that allow the reconstitution of the cellular interaction model of T1D. For T1D, as well as other polygenic disorders, iPSCs provide a novel starting point for establishing a relevant disease model of T1D onset and progression.

Challenges and Obstacles to iPSCs-Based Disease Modeling

hiPSCs-based human disease models face many of the same challenges as cell therapy. An important challenge is the lack of standard biomarkers for starting states and differentiated end states, enabling the recapitulation of disease phenotype in cells derived from patient-specific hiPSCs. Up to now, only a limited subset of disorders can be amenable to this reprogramming approach with a phenotype of any kind in the interested cell type. In most cases, differentiation to the adult fate has not been accomplished, and the painstaking work of developing appropriate cell culture or transplantation models for each disease will continue.

Preliminary data published on the modeling of monogenic and cell-autonomous diseases show that inherited diseases of high genetic penetrance with an early onset may be easier to model (5). In comparison, hiPSCs may be less effective as an *in vitro* system to study complex disorders, those in which gene–gene and gene–environmental interactions result in considerable variability of symptom presentation. Although there has been a substantial effort to generate disease-specific hiPSCs from patients with polygenic diseases, including Alzheimer's (61), Parkinson's disease (22), congestive heart failure, and diabetes (57), differentiation to a well-defined pathology has not been accomplished. In the study of age-dependent diseases such as Alzheimer's and Parkinson's disease, a particularly challenging obstacle to hiPSCs-based modeling is the substantial difference in kinetics of disease onset and progression occurring *in vitro*, since it takes years for symptoms to develop in patients. Thus, alternative strategies will have to be developed to facilitate disease pathogenesis in patient-specific hiPSCs, and to mimic epigenetic changes caused by aging and the environment. Another problem that needs to be addressed is whether such age-dependent diseases can be recapitulated *in vitro* within a few weeks or whether one needs to accelerate the phenotype by exposing cells to different types of environmental or genetic stress to unveil a phenotype.

Another important challenge is that many diseases develop in a non-cell-autonomous manner and may require the interaction of multiple different cell types. For instance, motor neurons alone derived from ALS patient-specific hiPSCs may not be able to reconstitute full disease pathogenesis, as they may need to interact with glial cells which carry superoxide dismutase (SOD) mutations (62,63). Even monogenic diseases, such as Duchenne muscular dystrophy (DMD), which is caused by dysfunction of the dystrophin gene, may be hard to model *in vitro* (8). When the hiPSCs cells generated from DMD patient are injected into Mdx mice blastocyst, mononuclear infiltration, global loss of utrophin, and changes in interrelated tissue (*i.e.* fat), may be involved in the potential non-cell autonomous role for dystrophin (64,65). Since any organ is not composed of just one cell type but several, all of the relevant cell types involved in disease may need to be reconstituted from patient-specific hiPSCs. Thus, three main factors may influence the amenability of diseases to *in vitro* modeling: the onset of disease in patients, the complexity of the underlying genetic defects and the cell-autonomy of the disorder (66). However, it is still unclear if these three elements differ for different diseases and cell types, and which of these three elements most strongly influences the ability to generate the relevant disease phenotype *in vitro*.

Although hiPSCs provide several advantages for disease modeling compared to hESCs, there are specific diseases for which hESCs might provide a better model. One example is Fanconi anemia (FA), which is caused by autosomal recessive or X-linked mutation in one of 13 genes in the FA pathway. Somatic cells from FA patient are resistant to reprogramming unless the gene defect is first corrected (9). Another strategy which employed gene knockdown in hESCs established a well-known disease model for investigators to research the pathological mechanisms of FA (67). FANCD2-knockdown hESCs demonstrated significant reduction in total colony-forming units; both FANCD2- and FANCA-knockdown hESCs were hypersensitive to DNA damage and showed a reduction in the ratio between gamma-globin and epsilon-globin, which can be rescued by overexpression of FANCD2 or FANCA gene. In this context, iPSCs fail to present a feasible strategy for the study of FA, while hESCs models derived by knocking down disease-specific genes can be used to gain greater insight into the FA pathophysiology.

Finally, diseases caused predominantly by epigenetic alterations may be difficult to study in iPSCs. Since somatic cells can be reprogrammed to hiPSCs by enforced expression of the four Yamanaka factors (OSKM), epigenetic alterations will persist in the hiPSCs, which may cause novel pathological variability to sporadic and multi-factorial disorders caused by a combination of genetic and environmental factors. Thus, hiPSCs from patients with significant epigenetic components may be of little value for mechanistic studies unless the epigenetic alterations associate with identified genetic alterations.

IPSCS IN DRUG DISCOVERY

Over the past decade, the pharmaceutical industry has witnessed attrition for a considerable number of drugs due to the emergence of toxicities and side effects in late-stage clinical trials, despite billions of dollars invested in research and development to improve the quality of pharmaceuticals produced. The efficient development of safe pharmaceuticals is impeded by the lack of appropriate predictive assay to evaluate drug toxicity. Efforts to identify novel compounds to resolve human disease phenotypes, and to accurately predict the toxicity profiles of drug compounds, have relied heavily on animal models, contributing to the high cost of drug development (68). Even so, many novel drug compounds have been less than successful in entering the market due to unanticipated hazardous effects exhibited in human clinical trials (69). Well known examples include the withdrawal of the anti-inflammatory drug Rofecoxib/Vioxx (Merck) from the market due to the high risk of cardiovascular side effects (70). Animal models, as described previously, do not accurately mirror human pathophysiology and hence their predictive value in drug development and toxicology assessment is limited. *In vitro* cell-based assays are typically used in early drug profiling studies and similar to animal models, their use in drug screening and predictive toxicology is inadequate (68). This calls for a greater need for improved cell-based assays and disease models for drug screening purposes. Such models may allow the identification of potentially toxic compounds for elimination during early stages of drug discovery processes, narrowing efforts to more promising candidates.

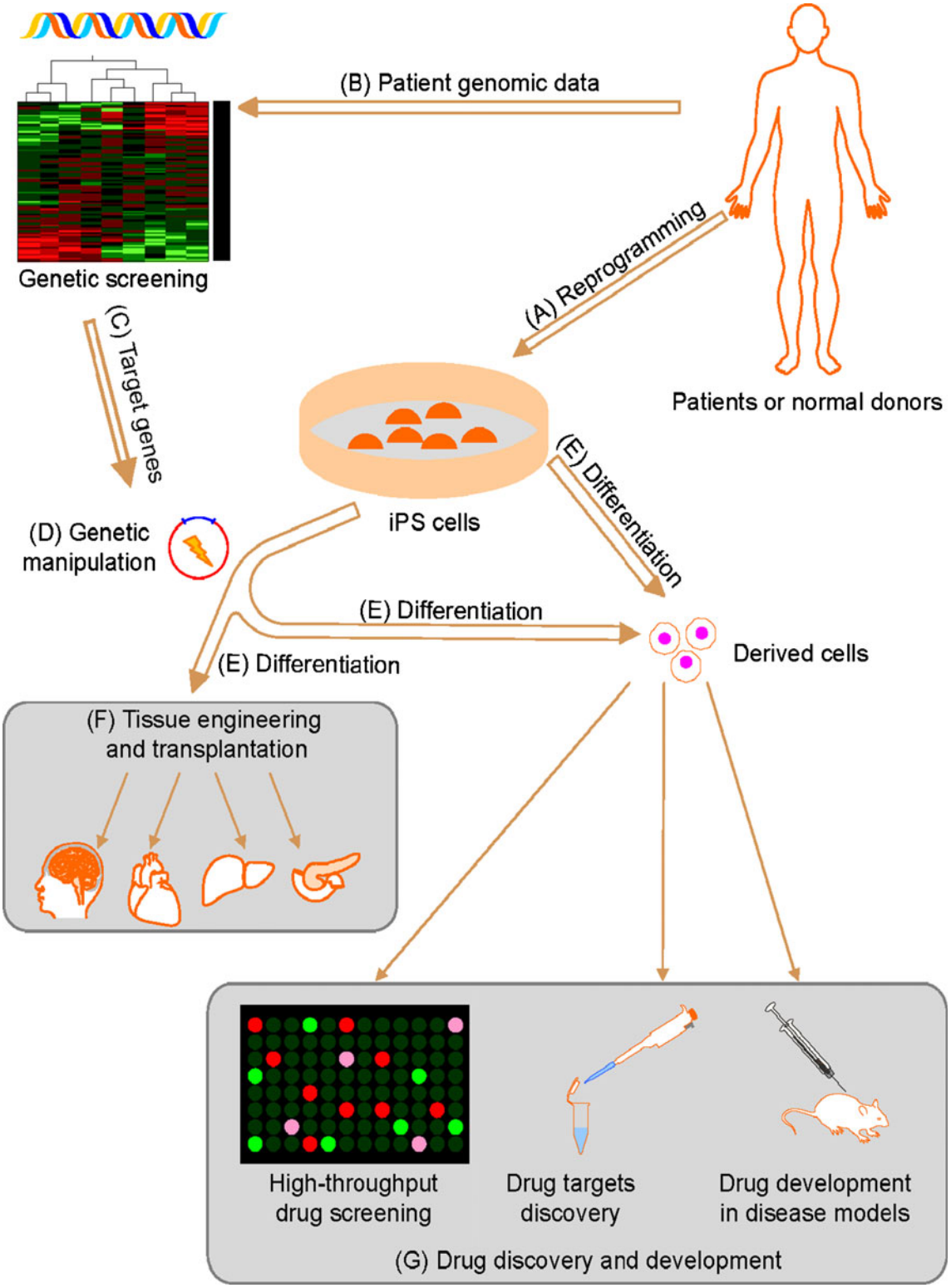
Human pluripotent stem cells and hiPSCs, may help to address the limitations of existing *in vitro* assays and animal models in drug discovery studies. The feasibility of obtaining patient-specific hiPSCs from readily available tissue samples empowers *in vitro* disease modeling and drug discovery processes, and at a relatively low cost. Since they retain all the genetic information of the patients, hiPSCs-based assays potentially enable the identification of patient subsets displaying specific adverse drug reaction profiles, and therefore provide novel approaches for demographic-based drug discovery and toxicology.

iPSCs in High-Throughput Chemical and Functional Screens

High-throughput screening technologies are widely used in the early stages of drug discovery to rapidly evaluate the properties of thousands of compounds. The availability of hiPSCs generated from patients with specific diseases offers exciting opportunities for ascertaining differential drug sensitivities through high-throughput screens. To date, there has yet to be any relevant high-throughput chemical screens performed using hiPSCs for discovering previously unidentified novel compounds to resolve human disease phenotypes; nevertheless, the field is poised for substantial advances. In combination with genome-wide association studies (GWAS)and our knowledge of disease-gene linkage, hiPSCs disease models may represent a novel biomedical interface, so as to expedite efforts in drug screening and development of personalized medicine (Fig. 1).

Since hiPSCs retains the genetic makeup of the patient and can be directly applied for drug screening, they represent a fast track approach to facilitate drug discovery, and enable the identification of compounds that might have a disproportional effect on certain patient populations. The proof-of-principle that hiPSCs present a viable platform for disease modeling and subsequent pharmaceutical testing is well-established: as previously mentioned, hiPSCs models successfully recapitulated the phenotype of spinal muscular atrophy (71), familial dysautonomia (72), Rett syndrome (73) and long QT syndrome (74,75), with the reversal of phenotypes observed by application of known drugs. Nevertheless, several obstacles have to be tackled before hiPSCs can be made available for high throughput chemical screening.

Disease phenotypes are typically observed only after patient-specific hiPSCs are differentiated into the specific disease cell types. Assuming that disease features can be reproduced *in vitro*, it is still unclear whether the phenotypes can be used for high throughput small molecules screening. The most critical challenges are whether the relevant disease phenotypes can be faithfully reproduced *in vitro* and, if so, whether they can accurately predict disease behavior *in vivo*. Despite several studies suggesting that certain features of familial dysautonomia, SMA and Rett syndrome can be generated using hiPSCs-derived neural cells, in certain diseases with a long latency period however, such as Huntington's or Parkinson's disease, it may be impractical to *in vitro* modeling these diseases with hiPSCs since they are unlikely to reflect the dynamics of disease progression occurring *in vivo*. In these cases, the appearance of phenotypes can be accelerated through the exposure of cells to environmental factors to promote aging (*i.e.* oxidative stress). A major challenge, therefore, would be to optimize robust lineage-specific differentiation protocols to generate sufficient quantities of purified cells of a specific type for large scale screening. A heterogeneous cell population consisting of distinct subtypes of differentiating cells is usually obtained, probably because different stem cells in a given hiPSCs line have different propensities to differentiate (76,77). This makes it harder to isolate desired cell types for screening, although this can potentially be solved by FACS sorting and other related methodology. In addition, hiPSCs-derived cells possess an embryonic and immature nature, and thus may exhibit vastly different clinical responses compared to adult cells in patients. For



instance, hiPSCs-derived hepatocytes have a fetal phenotype, lacking enzymes important in drug metabolism; this severely compromises drug screening for liver diseases (11). Finally, diseases with significant epigenetic components may be difficult to study in hiPSCs, given that reprogramming back to the pluripotent intermediate erases epigenetic components critical for the manifestation of certain diseases, impeding screening efforts (5,11).

◀ **Fig. 1** Applications of induced human pluripotent stem cells (hiPSCs) in pharmaceutical research and drug development. (**a**) Somatic cells from patients and/or normal donors are reprogrammed into hiPSCs through exogenous expression of reprogramming factors Oct4, Sox2, Klf4, and Myc (OSKM). With genome-wide screening from patient and control groups (**b**), target disease-associated genes (**c**) are identified and can be genetically engineered (through depletion/overexpression/mutagenesis) (**d**) in reprogrammed hiPSCs. The self-renewing wild-type and genetically-engineered hiPSCs can be expanded to large quantities, before they are induced with directional differentiation protocols (**e**) to produce cell lineages at later developmental stages (such as precursors and terminally differentiated somatic cells), which can then be used in cell-based therapies through tissue-engineering and transplantation (**f**). Moreover, these large-quantity hiPSCs are important tools for drug discovery and development (**g**), as they can be used to generate disease-specific cell lines and animal models for high-throughput drug screening, drug properties studies, and pre-clinical drug trials.

That being said, we strongly believe the hiPSCs platform serves as a powerful technology for future clinical trials. In certain diseases, a subset of patients carrying a specific genotype may react adversely or fail to respond to a given drug. Accordingly, hiPSCs can be derived from these patients and be used to study drug-genotype interactions for the development of novel drug derivatives and personalized medicine. We foresee a vast potential of hiPSCs to facilitate unbiased chemical screens for novel compound discovery.

In addition to its potential use in large scale chemical screens, hiPSCs cellular disease models may help to identify individual genetic components of certain diseases. Investigators can make use of tools to systemically perturb or overexpress genes in disease models *in vitro* in order to elucidate mechanisms of disease. Such genetic modification studies potentially allow the study of genetic mutants with observable phenotypes, to identify crucial factors involved in normal development and those associated with manifestation of diseases. Findings from these studies facilitate drug target validation and together allow the discovery of new therapeutics.

For instance, functional genomics approaches which can perturb hundreds of genes in parallel using libraries of siRNA directed against all the genes in the human genome have previously identified the ability of p53 siRNA to enhance efficiency of hiPSCs generation up to 100-fold, in the absence of Myc (78). Hence, using similar screening approaches such as short hairpin RNA (shRNA) libraries with hiPSCs cellular disease models may allow gain-of-function and/or loss-of-function analysis to illuminate molecular mechanisms underlying disease pathophysiology. In complex diseases, epistasis studies with the candidate modifiers genes could be performed using the same approach, to evaluate differences in phenotypic penetrance between individual patients (5). To facilitate such screening approaches, one could envision the engineering of specific lineage reporter cell lines from patient-specific hiPSCs (using GFP as readout of gene-of-interest expression of a diseased cell type). These reporter cell lines are particularly useful as they allow tracking and subsequent enrichment of specific cell types, since current *in vitro* directed differentiation protocols generate mixed cell cultures (14). These reporter cell lines can then be subjected to knockout screens using shRNA libraries or be directed for targeted genetic modification of specific loci to evaluate gene functions. One major disadvantage of using hiPSCs, however, is the genetic and epigenetic variability associated with most hiPSCs lines, and hence this calls for a need of appropriate controls when analyzing issues associating with genetic modification (5). The lack of proper control contributes a major impediment when it comes to defining a disease-relevant phenotype, and finally to the validation of specific drug target and development of novel compounds.

hiPSCs for Drug Toxicology Assessment

In addition to its potential uses in high throughput chemical and functional genomics screening, hiPSCs technology may serve as a powerful approach for predictive toxicology studies. This technique allows access to certain cell types that are impossible to obtain from actual humans, such as in hepatotoxicity, cardiotoxicity and neurotoxicity, which are further described below. Prior to the discovery of hiPSCs, mouse embryonic stem cells (mESCs) were used in an assay known as the mouse embryonic stem cell test (EST), which was employed by the pharmaceutical industry for evaluating a drug compound's cytotoxic, genotoxicity and inhibitory effect on the spontaneous differentiation of the mESCs into beating embryoid bodies (EBs) (79). This test was extensively applied in predictive teratogenicity. Despite validation studies reporting a relatively promising predictive efficiency of this assay compared to other *in vitro* models (*i.e.* correct classification of 78% of well-characterized compounds into the categories of non- embryotoxic, weakly embryotoxic and strongly embryotoxic compounds) (80), this test is limited in its ability to predict drug toxicity profiles for human diseases. Species-specific pharmacokinetic profiles frequently do not address the metabolism or the genetic diversity of patients living in heterogeneous conditions, thereby giving rise to drug-induced responses that may be clinically irrelevant in humans. Furthermore, mESCs-based assays may be inadequate to predict developmental toxicity of certain drugs which potentially affect developmental processes (81). To circumvent cross-species differences, an appropriate strategy would be to integrate hESCs or hiPSCs with molecular approaches for their use in toxicology assessment, not bounded by the prediction of teratogenicity alone. A recent encouraging work was demonstrating an improved efficiency of hESCs and metabolomics in predicting human developmental toxicity of drugs over

mESCs (82). It is likely that hiPSCs hold far greater promises for overcoming the limitations of current predictive toxicology tools and allow the rapid identification of environmental toxins.

Hepatotoxicity

Hepatotoxicity is a major concern in clinical drug development. Of note, drug-induced hepatotoxicity is shown to have the poorest correlation between regulatory animal toxicity and in humans, and further substantiating the imperfect extrapolation of cross-species findings to humans (83). Several mechanisms are involved in drug-induced hepatotoxicity, including steatosis, cholestasis, production of reactive intermediates and oxidative stress (84). In predictive hepatotoxicity assessment, hiPSCs can ideally be used as surrogate models to assay toxicity, for which the appropriate cell lines and/or models are lacking. For instance, although primary human hepatocytes are functionally and metabolically competent, and hence represent the closest *in vitro* model to intact human liver, their use in predictive liver toxicity studies is limited. They are difficult to be obtained and display significant intrinsic and phenotypic variability. In particular, they become metabolically incompetent when placed in culture (85). Transformed cell lines are also inappropriate for toxicology studies since they are genetically altered and may display phenotypic variability, giving rise to drug-genotype or drug-metabolism interactions that vary from those of primary human hepatocytes. hiPSCs-derived hepatocytes serve as physiologically relevant substitute for primary human hepatocytes and may provide further opportunities to study the role of genetic diversity in patients who exhibit idiosyncratic reactions to drugs (68). Nevertheless, as previously mentioned, current *in vitro* directed differentiation protocols face limitations in generating the desired cell populations, and the resulting hepatocyte-like derivatives often exhibit lower cytochrome P450 levels compared with the intact human liver (86). Further refinement of the differentiation protocols will be required for the rapid production of functionally and metabolically competent hepatocytes for use in drug-induced liver toxicity assessment.

Cardiotoxicity

hiPSCs-derived cardiomyocytes are advantageous in drug-induced cardiotoxicity studies, since they recapitulate *in vivo* cardiomyocyte functions, such as the hallmark electrophysiological properties and responsiveness to beta-adrenergic stimulation. Despite the embryonic nature of the cardiac action potential, similar expression of the ion channels as in the adult human heart is observed in these cardiomyocytes (87,88). An example of electrophysiological cardiotoxicity is commonly seen in drug-induced QT prolongation and proarrhythmia. This occurs when drug compounds interact and block the human potassium voltage-gated eag-related channel KCNH2, prolonging the ventricular action potential (89). The promising of hiPSCs as a physiologically relevant screening platform for cardiovascular drugs and in toxicology assessments can be seen in the following example. The plateau phase of the action potential in hiPSCs-derived cardiomyocytes was demonstrated to be shortened when treated with L-type calcium channel blocker nifedipine, but prolonged when treated with human KCNH2 blocker E-4031, as predicted in adult human cardiomyocytes (68). The remaining limitation lies in existing differentiation protocols as heterogeneous cell populations, consisting of ventricular-, atrial- and nodal-like cells are obtained (87,88).

On the other hand, mechanisms underlying another form of cardiotoxicity, known as biochemical cardiotoxicity, are poorly understood. Targeted cancer therapeutics in the small-molecule kinase inhibitor family may cause cardiotoxic side effects because they affect kinase pathways that are also expressed in the heart (90). Administration of imatinib (Gleevec) resulted in a dose-dependent decrease in cell viability and a concomitant increase in oxidative stress of hiPSCs-derived cardiomyocytes (68). Since hiPSCs-derived cardiomyocytes closely recapitulate most *in vivo* phenotypes and functions of cardiomyocytes, they may represent an ideal system for the assessment of both electrophysiological and biochemical cardiotoxicity

Neuronal Toxicity

As with cardiotoxicity and hepatotoxicity assessment, neuronal predictive toxicology may be assessed with *in vitro* models to allow better interpretation of neurotoxic responses in human. Testing in these *in vitro* toxicological models should ideally include cellular function tests such as electrical properties and alterations when exposed to toxic compounds, in addition to cell viability assays. A proof-of-principle demonstration that hESC-derived neuronal cells can be used for neurotoxicological screening has utilized a microelectrode array platform (91). This enables the measurement of neuronal network function for long periods of time *in vitro*. With recent significant progress in the cellular reprogramming field, marked advances for the application of hiPSCs in neurotoxicological screening is expected in the near future.

In summary, hiPSCs technology would provide a novel and standardized source of differentiated human cells for drug screening assays, and would substantially decrease the risk and cost associated with early stage clinical trials toward a more personalized approach in drug administration.

Tissue Engineering with hiPSCs Derivatives for Drug Screening and Toxicology Assessment

Despite the vast potential of hiPSCs in disease modeling and drug discovery, it is naïve and impractical to attempt to recapitulate most human disease phenotypes with a single hiPSCs-derived, lineage-committed cell type, since disease pathology is likely to arise from the interaction of the diseased cell type with other relevant cell types, as previously described. In ALS for example, cocultures of wild-type, hESC-derived motor neurons with mutant ALS astrocytes induced motor neuronal death (54,55). Since biological functions might be influenced by both cell-autonomous and non-cell-autonomous stimulation (*i.e.* neighboring cells, soluble factors, extracellular matrix and microenvironmental stimuli) (14), it is imperative to reconstruct such interactions within tissues or between organs, and to closely or fully recapitulate tissue architecture for effective *in vitro* disease modeling.

Cell culture systems and organoids may provide a richer context for studying disease relevant cell-cell interactions (5,14). These contextual cues are particularly important for modeling non-cell-autonomous pathology. In addition, advances in microscale engineering technologies enabled the development of cell-based biochips to reconstitute tissue architecture retaining the structural and mechanical features of living organ systems. These biomimetic microsystems enable manipulation and control of the cellular microenvironment with high spatiotemporal precision, equip the study of complex interactions between living tissues and an organ in a physiologically relevant context, and provide a new type of platform for bridging the gap between *in vitro* assays and animal studies (92–95). This "organ-on-a-chip" technology may potentially replace costly and time-consuming animal testing studies, which contribute to the high cost of pharmaceutical development. Marked advances in the development of such biomimetic microsystems would surely revolutionize many important fields, particularly in the areas of pharmaceutical development, toxicology assessment and personalized medicine. Moreover, we envision the implications of hiPSCs applied in the organ-on-a-chip technology. This enabling technology brings a higher order complexity to hiPSCs-based drug discovery. In this case, hiPSCs would serve as a source of patient-specific, hiPSCs-differentiated derivatives, which could be cultured and assayed in the biochip. Ideally, the microsystem would also comprise of hiPSCs derivatives cultured with known interacting tissue types in a physiologically relevant microenvironment to recapitulate the disease phenotype, and subsequently be applied for drug screening and toxicology studies.

A proof-of-principle for the biomimetic microsystems approach was established by the Ingber group at the Wyss Institute for Biologically Inspired Engineering. They designed a microdevice that reportedly reconstitutes the critical structural, mechanical, and physiological properties of the human lung alveolar-capillary interface (96). Accordingly, this human-breathing lung-on-a-chip initiates complex, whole-organ responses to bacteria and inflammatory cytokines introduced to the alveolar surface. This group also demonstrated that breathing motions and mechanical stress might greatly accentuate toxic and inflammatory responses of the lung to silica nanoparticles (96).

Other biomimetic organ models that could have high-impact applications as well (97). A more predictive human kidney-on-a-chip, for instance, would be a valuable tool for investigating ADMET (absorption, distribution, metabolism, excretion and toxicological) properties of new chemical entities during drug development. Another desirable tool is the human intestine-on-a-chip that exhibits peristalsis. The intestine is the major site for absorption of orally-delivered drugs, foods and nutriceuticals. Intestinal epithelial cells have drug metabolizing enzymes and drug transporters that play critical roles in determining ADMET of various chemical compounds that influence bioavailability and potential drug–drug interactions.

The implications of these findings are far-reaching, providing further opportunities to model critical tissue-tissue interfaces accurately and for the input of these novel biomimetic microsystems in combination with patient-specific hiPSCs into high-throughput screening of cellular responses to chemicals, drugs and various environmental toxins.

USES OF HIPSCS IN REGENERATIVE AND PERSONALIZED MEDICINE

The implications for the use of patient-specific hiPSCs in transplantation therapies are astounding and serve to revolutionize the field of regenerative medicine. As previously described, a substantial advantage is the limitation of ethical issues and potential elimination of the threat of transplant rejection when utilizing patient-specific hiPSCs-derived cells to replace tissue loss from disease, injury and aging, as opposed to using ESCs derivatives. The developmental potential of hiPSCs and their potential for disease treatment have been demonstrated in recent studies performed using murine models. Although the use of hiPSCs as a platform for the development of therapies is just beginning, we highlight several examples that describe the considerable progress driving patient-specific hiPSCs for uses in regenerative medicine.

It was shown by Hanna and colleagues that a humanized mouse model of sickle cell anemia can be rescued after

transplantation of autologous iPSCs-derived hematopoietic progenitors (98); Wernig and colleagues demonstrated the reprogramming of murine fibroblasts into iPSCS followed by *in vitro* neutralization to generate multipotent neural precursor cells (NPCs) capable of producing neurons, astrocytes and oligodendrocytes (99). Establishing a proof-of-concept that iPSCs can be applied in transplantation therapy to restore physiological function of the diseased tissue *in vivo*, the authors further performed transplantation of the NPCs into a Parkinsonian rat model. Direct injection of the NPCS into the midbrain gave rise to the migration and subsequent differentiation of the iPSCs-derived NPCs into neurons and glia, followed by production and integration of dopaminergic neurons in the host, leading to functional recovery. In human neuron regenerative medicine research, transplantation of *in vitro* produced hESC-derived dopaminergic neurons into Parkinsonian rats showed functional engraftment of the cells to restore dopamine function (100). Using standardized neutralization protocols, Parkinson patient-specific hiPSCs could also be directed for *in vitro* dopaminergic neuron generation (53). Moreover, even though iPSCs-derived neuronal cells have not been used for transplantation in an experimental spinal cord injury model, they hold great promise for restoring physiological function following neurotrauma (101). For instance, the pathological hallmark of neurotrauma or spinal cord injury is demyelination, resulting in vulnerable axons and impaired CNS function. One can envision the use of patient-specific hiPSCs to regenerate damaged tissues in spinal cord injury: patient-specific hiPSCs can be directed for differentiation into NPCS, and the resulting cells can be injected directly into the injured spinal cord. Ideally, these transplanted cells can differentiate into the various CNS cellular subtypes, contributing to functional recovery.

In summary, the application of hiPSCs in regenerative medicine is still a long-term project which would require the engagement of regulatory bodies for rigorous examinations of its safety, and optimization of the protocols required to differentiate patient-specific hiPSCs to the desired cell types for transplantation purposes.

CONCLUDING REMARKS

hiPSCs technology is emerging as an unprecedented opportunity in pharmaceutical research, biomedical research, disease modeling, drug discovery and regenerative medicine. However, our basic knowledge of hiPSCs generation is still in early stages. To harness the full potential of this technology, issues regarding the standardization of hiPSCs, criteria to define hiPSCs and technological development for their standardization still need to be addressed. In particular, clones with different differentiation abilities obtained in the process of generating hiPSCs require basic verification, such as the difference between the clones and the way to control the changes within one clone over culturing and successive subculture.

Standardization of hiPSCs repository with a collection of various types of hiPSCs is urgently needed. The objective of an hiPSCs bank would be to increase the number and quality, preservation and distribution of hiPSCs lines available for activities such as *in vitro* disease modeling and high-throughput screening. In November 2010, the California Institute for Regenerative Medicine (CIRM) organized a workshop with leaders in the field of stem cell research and regenerative medicine to assess the value of supporting more formal hiPSCs banking efforts. Another useful resource for researchers studying neurodegenerative disorders interested in patient-specific fibroblasts or hiPSCs lines is the National Institute of Neurological Disorders and Stroke (NINDS)/Coriell Cell bank repository; this repository receives, standardizes the collection, and stores of hiPSCs lines generated from patients of PD, ALS, and HD. The goal of this consortium is to generate hiPSCs lines from all familial forms of the 3 major neurodegenerative disorders, with the inclusion of cell lineage-specific reporter lines associated with each disorder. The Harvard Stem Cell Institute (HSCI) iPSCs core is also cataloguing lines produced by HSCI scientists, and provides a service to produce disease-specific lines. The rigorous efforts undertaken are encouraging and reflect the positivity of the scientific community's attitude towards the potential of hiPSCs, allowing the technology to gain rapid advances.

Despite their vast potential in pharmaceutical and biomedical research, concerns over the use of hiPSCs in disease modeling and subsequent clinical applications have emerged. Due to the technical limitations of current reprogramming protocols, hiPSCs have imperfect clearing of epigenetic memory of the original cell type, hence impeding differentiation to the desired cell type of a different lineage (102,103). In addition, incomplete reprogramming inherently produces heterogeneous cell populations containing both bona fide hiPSCs and other incompletely reprogrammed intermediates, which may interfere with *in vitro* assays of disease and drug screening (15). Prolonged growth in cell culture may produce chromosomal abnormalities in hiPSCs hence karyotypic analysis should be frequently performed on hiPSCs and their differentiated derivatives (104). Furthermore, due to our incomplete understanding of the reprogramming process, there are significant differences underlying between hESCs and hiPSCs. As previously described, hiPSCs-based disease modeling may not prove satisfactory for all diseases, and face significant challenges to get an effective *in vitro*

modeling. Unless future work enables the full reprogramming of cells, hESCs might be a better choice for some types of disease modeling. Lastly, significant work must be invested to rigorously demonstrate that *in vitro* directed reprogramming and/or differentiation protocols can produce cells that are functionally equivalent to those produced during normal development *in vivo*.

The success of the hiPSCs-based disease modeling field greatly depends on the efficient development of consistent protocols enabling cellular reprogramming or directed differentiation to the desired cell types in quantities sufficient for pharmaceutical research and clinical applications, and the translation of current protocols to befit the therapeutic system. As the field of hiPSCs-based disease modeling expands, we can expect promising results that will deepen our knowledge on cellular reprogramming, further our understanding of disease mechanisms, and provide better treatment options to patients.

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