

Review

iPSCs and small molecules: a reciprocal effort towards better approaches for drug discovery

Ru ZHANG¹, Li-hong ZHANG², Xin XIE^{1,2,*}

¹Laboratory of Receptor-based Bio-medicine, Shanghai Key Laboratory of Signaling and Disease Research, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China; ²CAS Key Laboratory of Receptor Research, the National Center for Drug Screening, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

The revolutionary induced pluripotent stem cell (iPSC) technology provides a new path for cell replacement therapies and drug screening. Patient-specific iPSCs and subsequent differentiated cells manifesting disease phenotypes will finally position human disease pathology at the core of drug discovery. Cells used to test the toxic effects of drugs can also be generated from normal iPSCs and provide a much more accurate and cost-effective system than many animal models. Here, we highlight the recent progress in iPSC-based cell therapy, disease modeling and drug evaluations. In addition, we discuss the use of small molecule drugs to improve the generation of iPSCs and understand the reprogramming mechanism. It is foreseeable that the interplay between iPSC technology and small molecule compounds will push forward the applications of iPSC-based therapy and screening systems in the real world and eventually revolutionize the methods used to treat diseases.

Keywords: induced pluripotent stem cells (iPSCs); disease modeling; drug screening; toxicity evaluation; cell replacement therapy; small molecules; drug development

Acta Pharmacologica Sinica (2013) 34: 765–776; doi: 10.1038/aps.2013.21; published online 22 Apr 2013

Introduction

The development of human society is always accompanied by the evolution of diseases. Many diseases, especially degenerative disorders, still lack effective cures. In recent decades, the progress in stem cell research has provided new hope for cell replacement therapies and new methods to identify drugs. Stem cells can be roughly separated into two categories. The first type, adult stem cells, or organ-specific stem cells, generally have limited potential for growth and differentiation, whereas the second type, embryonic stem cells (ESCs), can be cultured *in vitro* indefinitely and can theoretically give rise to all cell types. The recent discovery of induced pluripotent stem cells (iPSCs) has created even more enthusiasm for exploring the therapeutic potential of stem cells in regenerative medicine because iPSCs overcome the ethical and histocompatibility issues hampering the use of human ESCs^[1,2].

In contrast to human ESCs, iPSCs can be generated with direct reprogramming of adult skin cells (or other easily obtainable adult cells) with defined transcription factors (Oct4, Sox2, Klf4, c-Myc, *etc*), indicating that every person could have

his/her own iPSCs^[1–3]. iPSCs derived from patients, especially patients carrying disease-related genetic mutations, should retain these mutations and be able to give rise to cells that harbor the disease phenotype^[4–8]. These disease-carrying iPSCs could be genetically corrected *in vitro*, differentiated into the appropriate cell types, and transferred back into patients to support normal physiological functions^[6,7], or these disease-carrying cells could be used as *in vitro* models to study the mechanisms of the disease or serve as tools to screen for new small-molecule drugs^[4,5,8].

On the other hand, the existing small-molecule drugs also have helped to advance iPSC technology and dissect the underlying mechanisms of somatic cell reprogramming. Although iPSCs have enormous potential, the clinical application of iPSC technology is still hindered by safety concerns and relatively low efficiency. To prevent the use of oncogenes and the incorporation of viral DNA sequences, considerable effort has been expended to modify the original reprogramming protocols by using a reduced number of factors^[9], non-integrating gene delivery approaches^[10], and cell-permeable proteins to trigger the reprogramming^[11]. Small molecules, due to their ease of use and structural versatility, have attracted considerable interest for steering reprogramming towards a faster, more efficient, and directed process.

* To whom correspondence should be addressed.

E-mail xxie@mail.shcn.ac.cn

Received 2012-12-31 Accepted 2013-02-07

In this review, we discuss the therapeutic potential of iPSCs, focusing on iPSC-based cell therapy, disease modeling and drug evaluations. In addition, we discuss the use of small-molecule drugs to improve the generation of iPSCs and understand the reprogramming mechanism. Hopefully, the interplay between iPSC technology and small-molecule compounds will facilitate the development of new therapies.

iPSC-based cell therapy

One of the most exciting aspects of iPSC technology is the possibility of generating autologous cells for cell-replacement therapy. The somatic origin of iPSCs has minimized the ethical concerns and greatly reduced the immune rejection problems that have hampered the development of human ESC-based therapies. Hanna and colleagues^[6] demonstrated for the first time that iPSCs derived from a humanized mouse model of sickle cell anemia could be genetically corrected, differentiated into hematopoietic progenitor cells *in vitro*, and then transplanted back into the original mice, resulting in an improved phenotype. Raya *et al* generated genetically corrected iPSCs from patients with Fanconi's anemia (FA), and the hematopoietic progenitor cells derived from these iPSCs demonstrated functional FA pathway reestablishment *in vitro*^[7].

Similar cell-replacement strategies have been reported for other organs and implemented in many other disease models. For example, Wernig and colleagues^[12] successfully derived functional dopaminergic neurons from iPSCs, and the transplantation of these neurons into a rat model of Parkinson's disease (PD) resulted in normalized dopamine activity and alleviation of PD symptoms. Neurospheres produced by iPSCs have been transplanted into the brain of a spinal-cord injury mouse model, and functional recovery was confirmed with accompanying re-myelination and axonal regrowth^[13]. Transplantation of factor VIII (FVIII)-producing endothelial cells derived from iPSCs has been reported to rescue mice carrying hemophilia A (caused by a mutation in FVIII) from a death-inducing bleeding assay^[14]. In addition, iPSCs have also been differentiated into functional cardiac myocytes^[15], and human-iPSC-derived cardiac myocytes have been shown to improve the cardiac contractile function in the experimentally injured rodent heart^[16, 17]. Mouse iPSC-derived mature β cells generated from chimeric animals were used to rescue syngeneic diabetic mice induced with streptozotocin^[18]. Insulin-producing cells have also been generated from human iPSCs^[19], and the function of these cells has been confirmed in a mouse model of type I diabetes^[20].

Although the therapeutic effects of stem cells have been proven in many animal models, the ESC or iPSC-based cell therapy is still hindered by safety concerns regarding tumor formation and technical difficulties in generating large quantities of pure, well-differentiated and functional cells. Nevertheless, the generation of differentiated cells or tissues from patient-specific iPSCs may provide a new approach for disease modeling and drug screening *in vitro*.

iPSC-based disease modeling and drug screening

The average time required to develop a new drug is between 10 and 15 years, and the associated costs can reach one billion USD^[21]. More than 90% of the drugs tested in clinical trials fail to be approved because of a lack of sufficient efficacy or unanticipated toxicity^[22]. Discovering new effective entities and evaluating their safety are two key aspects of developing new drugs. The success of preclinical phases of drug development is largely based on animal models, which might fail to translate into real human diseases. Disease or screening models that faithfully represent actual human diseases or human physiology are needed to improve the success rate of drug discovery and development. Drug discovery/development platforms using iPSC-based disease models could be useful in filling the gap between animal models and clinical trials.

Several hurdles must be overcome to apply iPSCs to drug discovery. First, iPSCs must be generated from nonpluripotent patient cells, and then, these patient specific iPSCs have to be differentiated into the mature cells that express the disease-relevant cellular pathology, a key step and major challenge in "disease modeling". Based on these disease phenotype-expressing cells, we can screen for novel compounds that might correct the cellular pathology or evaluate possible toxicity of drugs. Although theoretically appealing, modeling diseases that are more genetically complex, such as sporadic Alzheimer's disease (AD), remain challenging. To date, only partial disease modeling has been reported, mostly for monogenic disorders. Nevertheless, these not-so-perfect disease models could still be important and relevant, especially in situations when animal models do not recapitulate the actual human diseases.

Neurological diseases

Neurological disorders account for 6.3% of the global burden of diseases^[23], and this burden is expected to increase as the human life span is extended. Most neurological disorders are caused by the dysfunction or loss of specific populations of neuronal and/or glial cells. As human neurons and glia are not readily available, studies have been traditionally limited to genetically engineered animal models or cell lines less relevant to the disease pathophysiology. The introduction of iPSC technology has provided a new approach for the study of neurological disorders at a cellular level.

Spinal muscular atrophy (SMA), familial dysautonomia (FD) and Rett syndrome (RS) are early onset monogenic disorders that provide a relatively easy start point for iPSC-based disease modeling. SMA is the most common neurogenetic disorder of infancy. The disease is caused by deletions of the *SMN1* gene, resulting in a decreased level of survival of motor neuron (SMN) protein, which eventually leads to the loss of motor neurons and the consequent denervation of the axial and limb muscles. This loss manifests clinically as muscle atrophy and weakness, dysphagia and respiratory failure in severe cases^[24]. Researchers have screened for compounds that elevate SMN levels in various engineered cell lines and patient fibroblasts,

but these compounds have failed in the clinic possibly because the mechanisms that regulate SMN protein levels in fibroblasts or engineered cells are substantially different from those in human motor neurons *in vivo*. Ebert and colleagues created two iPSC lines^[25] and demonstrated that SMA motor neurons derived from iPSCs developed normally but were more susceptible to degeneration accompanied by a reduction in SMN aggregates. Two drugs, valproic acid (VPA) and tobramycin, have been found to increase the number of SMN-rich structures in the SMA iPSCs. Although it remains to be seen whether these compounds can have the same effect in patient motor neurons, these early studies provide an important validation of the utility of iPSC-derived patient cells to model the disease.

FD is caused by mutations in the *IKBKAP* gene^[26] that lead to the abnormal migration of neural crest cells and results in the loss of sensory, sympathetic and parasympathetic neurons. In a recent study, Lee and colleagues derived iPSCs from three young patients with FD and induced the differentiation of these iPSCs into neural crest cells^[4]. Several disease-related defects were confirmed in these neural crest cells: a splicing defect in *IKBKAP*, a cellular migration defect and a defect in neurogenesis. The researchers also reported that the small molecule kinetin markedly reduced the splicing defect and modestly affected neurogenesis but had no effect on cell migration. Gene expression profiling of the FD neural crest cells also identified genes involved in peripheral neurogenesis and neuronal differentiation, providing insight into the molecular mechanisms of the disease. This study confirmed the application of iPSC-based cell models in phenotypic drug screens and for the identification of novel mechanisms and molecular targets.

Another developmental neurological disease, RS, is caused by the mutations in methyl-CpG-binding protein 2 (MeCP2), a protein involved in DNA methylation^[27]. iPSCs from RS patients have also been generated and differentiated into GABAergic neurons^[5]. Although no obvious changes in neurogenesis were observed, a reduction in the number of synaptic spines, a phenomenon observed in the post-mortem brains of RS patients, was reported. Calcium oscillations and the frequency of spontaneous postsynaptic currents were also decreased in neurons derived from RS iPSCs, suggesting a decreased neuronal networking activity. Using these cells, the authors also identified compounds that partially rescued the disease phenotype. Another study from the same group demonstrated that neural cells derived from RS iPSCs manifest increased L1 transposon motility, providing another mechanism that may underlie the disease and a novel target for drug discovery^[8].

Amyotrophic lateral sclerosis (ALS) and PD are late-onset neurological disorders. ALS is a fatal motor neuron disease, characterized by the progressive loss of motor neurons and muscle atrophy. Only approximately 10% of the patients have a genetic etiology, which makes modeling the disease more difficult. To date, several iPSC lines have been established from ALS patients carrying a SOD1 point mutation^[28, 29], and

functional motor neurons have been generated. However, unfortunately, the ALS-specific phenotype has yet to be determined. PD, the second most common neurodegenerative disorder, is caused by the progressive loss of dopaminergic neurons in midbrain. Although the symptom can be initially controlled, the progressive neurodegeneration will eventually lead to the loss of therapeutic efficacy. iPSCs have been established and used for *in vitro* disease modeling for two types of familial PD. iPSCs carrying the mutation in the gene encoding leucine-rich repeat kinase 2 (LRRK2), the most common cause of familial PD, were derived by Nguyen *et al*^[30]. The iPSC-derived dopaminergic neurons displayed increased α -synuclein level and sensitivity towards environmental stress. Dopaminergic neurons generated from iPSCs carrying mutations in the gene encoding PTEN-induced putative kinase 1 (PINK1) exhibited impaired recruitment of Parkin, another protein associated with familial PD and mitochondrial dysfunction that could be corrected by introduction of wild-type PINK1^[31]. It remains to be determined whether these anomalies also exist in dopaminergic neurons derived from patients with sporadic PD and thus providing common mechanisms and targets for drug discovery and development.

Disease modeling based on iPSC technology has also been applied to many other neurological or psychiatry disorders, including Alzheimer's disease^[32], Huntington's disease^[33, 34], Angelman and Prader-Willi syndromes^[35, 36], schizophrenia^[37], and others (Table 1).

Cardiovascular diseases

Heart disease, caused by both environmental and genetic factors, is the leading cause of death in many countries. More than 40 cardiovascular disorders, including hypertrophic cardiomyopathy, inherited arrhythmias such as familial long QT syndrome (LQTS), short QT syndrome and conduction system disorders, are caused by single gene mutations^[38]. Similar to neurological disorders, the limited availability of human cardiomyocytes is a major obstacle for replacement therapy and drug discovery and development. Many studies are still relying on the use of animal models and non-human cardiomyocytes. Human iPSC-derived cardiomyocytes offer an unprecedented opportunity to establish patient-specific lineages for disease modeling and drug screening. To date, both hypertrophic cardiomyopathy and cardiac channelopathy have been modeled using cardiomyocytes derived from patient iPSCs.

Carvajal-Vergara and colleagues^[39] generated two iPSC lines from two patients with LEOPARD syndrome (LS), who carry a heterozygous T468M mutation in *PTPN11* (protein tyrosine phosphatase, non-receptor type 11). LEOPARD is the acronym for its main features: lentigines, electrocardiographic abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormal genitalia, retardation of growth and deafness. Among these features, hypertrophic cardiomyopathy is the most life threatening. Interestingly, *in vitro* differentiated cardiomyocytes from LS iPSCs are larger and have a higher degree of sarcomeric organization and nuclear localization of NFATC (nuclear factor of activated T-cells, cytoplasmic,

Table 1. Summary of iPSC-based disease modeling and drug evaluation.

| Disease type | Disease name | Genetic cause | iPSCs differentiated to: | Phenotype | Drug test | Ref |
|-------------------------|---|--|--|---|--|------|
| Neurological | Alzheimer's disease | Polygenic (including mutation in <i>PS1</i> and <i>PS2</i>) | Neurons | Increase A β 42 secretion | γ -Secretase inhibitors and modulators blocked A β 42 secretion | [32] |
| | Amyotrophic lateral sclerosis | Polygenic (including <i>SOD1</i> mutation) | Motor neurons | ND | ND | [29] |
| | Angelman syndrome and Prader-Willi syndrome | Polygenic (chromosome 15q deletion, imprinting disorders) | Disease iPSCs obtained | ND | ND | [35] |
| | Down syndrome | Monogenic | Disease iPSCs obtained | ND | ND | [33] |
| | Familial dysautonomia | Monogenic (<i>IKBKAP</i> mutation) | All three germ layers including neural crest cells | Splicing defect in <i>IKBKAP</i> , cellular migration defect and neurogenesis defect | Small molecule kinetin reduced the splicing defect and modestly affected neurogenesis | [4] |
| | Fragile X syndrome | Monogenic | Disease iPSCs obtained | Loss of FMR1 expression | ND | [95] |
| | | | | Changes GAA-TTC repeat | ND | [96] |
| | Friedreich ataxia Huntington's disease | Monogenic (CAG repeat expansion in <i>Huntingtin</i> gene) | Neuronal precursors and striatal neurons | Contain same CAG expansion, enhanced caspase activity upon growth factor deprivation | ND | [33] |
| | | | | Neurons | Significant increase in lysosomal activity | ND |
| | Parkinson's disease | Polygenic | Dopaminergic neurons | No obvious defect | ND | [98] |
| | | Polygenic (including <i>PINK1</i> mutation) | Dopaminergic neurons | Impaired recruitment of Parkin, dysfunction of mitochondria | ND | [30] |
| | RETT syndrome | Monogenic (<i>MeCP2</i> mutation) | Neural progenitor cells and functional neurons | Loss of synapses, reduced spine density, smaller soma size | IGF1 increased glutamatergic synapse number; gentamicin increased MeCP2 protein level and synapse number | [5] |
| | Schizophrenia | Polygenic | Neurons | Reduced neurite density, neuronal connectivity and glutamate receptor expression | Loxapine increased neuronal connectivity and glutamate receptor expression | [37] |
| Spinal muscular atrophy | Monogenic (<i>SMN1</i> deletion) | Neurons and astrocytes, mature motor neurons | Loss of <i>SMN1</i> gene expression, susceptible to degeneration | Valproic acid and tobramycin increased the number of SMN-rich structures | [25] | |
| Cardiac and vascular | Arrhythmogenic right ventricular cardiomyopathy | Polygenic (including <i>PKP2</i> mutation) | Cardiomyocytes | Cardiomyocytes enlarged and contained more lipid content, reduced expression of <i>PKP2</i> and plakoglobin | ND | [46] |
| | Brugada syndrome | Polygenic (including <i>SCN5A</i> mutation) | Cardiomyocytes | Prolonged action potential, decreased current density | ND | [45] |
| | Catecholaminergic polymorphic ventricular tachycardia | Polygenic (including <i>CASQ2</i> mutation) | Cardiomyocytes | Immature phenotype with less organized myofibrils, abnormal response towards β agonist | ND | [47] |
| | Long QT 1 syndrome | Monogenic (<i>KCNQ1</i> mutation) | Cardiomyocytes | Prolonged action potential duration | ND | [41] |
| | Long QT 2 syndrome | Monogenic (<i>KCNH2</i> mutation) | Cardiomyocytes | Prolonged action potential duration, reduction of the cardiac potassium current | Used to evaluate the effect of several ion channel blockers as anti-arrhythmic agents | [42] |
| | Timothy syndrome | Monogenic | Cardiomyocytes | Increased cardiomyocyte depolarization | Roscovitine restored the electrical and Ca ²⁺ signaling properties | [44] |

(To be continued)

| Disease type | Disease name | Genetic cause | iPSCs differentiated to: | Phenotype | Drug test | Ref |
|-----------------|---------------------------------------|--|--------------------------|--|--|-------|
| | LEOPARD syndrome | Monogenic (<i>PTPN11</i> mutation) | Cardiomyocytes | Increased cardiomyocyte size, higher degree of sarcomeric organization and nuclear localization of NFATC | ND | [39] |
| Others diseases | A1-antitrypsin deficiency | Monogenic | Liver cell | Loss of A1-antitrypsin expression | ND | [53] |
| | Diabetes (Type 1) | Polygenic | Insulin-producing cells | ND | ND | [19] |
| | Diabetes | Polygenic (with mitochondrial DNA A3243G mutation) | Disease iPSCs obtained | ND | ND | [99] |
| | Fibrodysplasia ossificans progressiva | Monogenic (<i>ALK2</i> mutation) | Disease iPSCs obtained | Constitutive activation of <i>ALK2</i> , blocking reprogramming | <i>ALK2</i> inhibitors rescued reprogramming | [100] |
| | Polycystic kidney disease 1 | Monogenic (<i>Pkd1</i> deletion) | Disease iPSCs obtained | iPSCs underwent auto-correction by mitotic recombination | ND | [101] |

ND: not determined.

calcineurin-dependent).

LQTS, a common type of cardiac arrhythmia, is mainly caused by mutations of the genes encoding ion channels. Mutations in the voltage-gated K⁺ channels genes, including *KCNQ1* and *KCNH2*, lead to type 1 and type 2 LQTS, respectively, whereas mutations in the voltage-gated Na⁺ channel gene *SCN5A* lead to type 3 LQTS^[40]. Moretti *et al* generated cardiomyocytes from iPSCs derived from patients with familial type 1 LQTS carrying a R190Q mutation in the *KCNQ1* gene^[41]. These cells exhibited prolonged action potential duration and were able to recapitulate the electrophysiological phenotype of this disorder. Two studies have demonstrated the successful generation of iPSCs from type 2 LQTS patients carrying A614V or G1681A mutations in the *KCNH2* gene^[42, 43]. Cardiomyocytes differentiated from these iPSCs displayed significant prolongation of the action potential duration; this phenomenon was caused by a reduction of the cardiac potassium current. These cells have also been used to evaluate the effect of several ion-channel blockers as anti-arrhythmic agents. An iPSC-based model of LQTS in Timothy syndrome (TS) patients has also been reported. TS is caused by a mutation in the gene encoding the main L-type channel in the mammalian heart^[44]. Cardiomyocytes from these iPSCs displayed the molecular hall marks of LQTS, and treatment with roscovitine restored their electrical and Ca²⁺-signaling properties.

Disease-specific iPSCs have also been generated from other heart diseases such as Brugada syndrome^[45], arrhythmogenic right ventricular cardiomyopathy^[46] and catecholaminergic polymorphic ventricular tachycardia^[47] (Table 1). Similar to the studies focused on neurological disorders, these studies not only support the use of the iPSC-derived cardiomyocytes to model the abnormal functional phenotype of inherited cardiac disorders *in vitro* but also demonstrate the possibility of using these cells to screen existing or experimental drugs. However, the modeling of adult-onset diseases remains a challenge for iPSC-derived cells because aging or environmentally

triggered diseases may be mediated by epigenetic modifications of certain genes, and these modifications are reset with current iPSC technology.

iPSC-based toxicity evaluation

The attrition rate of drugs in development for humans, much of which can be attributed to unforeseen toxic side effects, mainly cardiotoxicity and hepatotoxicity^[48] that were not observed in preclinical animal models, is a significant problem facing the pharmaceutical industry. Approximately 30% of the medicines that have entered clinical trials were abandoned because of a lack of efficacy and another 30% were halted because of safety concerns^[49]. Predictive *in vitro* toxicity assays with adequate accuracy would help to reduce the failure rate and thus the cost of drug development. The use of human iPSCs and iPSC-derived cardiomyocytes and hepatocytes should be able to provide straightforward assays for analyzing many aspects of drug metabolism and toxic side effects.

The heart has been proven to be particularly sensitive to off-target, life-threatening toxic effects of non-cardiac drugs. Over the past decade, unexpected drug-induced ventricular arrhythmias associated with sudden cardiac death has led to the withdrawal of a number of drugs from the market^[50], among them the famous non-steroidal anti-inflammatory drug Vioxx from Merck. The blockade of the ether-a-go-go-related gene 1 (*hERG1*, also named *KCNH2*) channel is one of the major causes of drug-induced LQTS. Though it is still difficult to obtain pure mature cardiomyocytes for drug toxicity studies, significant progress has been made. Normal responses towards β-adrenergic and muscarinic stimulations have been demonstrated in human iPSC-derived cardiomyocytes by several studies^[17, 51, 52], indicating normal functions of these cells. It is foreseeable that in the near future, cardiac toxicity screenings based on these cells will soon become a valuable tool for the early evaluation of drug toxicity.

For drug metabolism and toxicity studies, primary human

hepatocytes are still the golden standard of *in vitro* models, as they express the full spectrum of drug metabolizing enzymes and transporters. However, the availability of primary human hepatocytes and batch-to-batch variation can lead to unnecessary complications. Human iPSC-derived hepatocytes will not only provide a scalable supply of cells for general metabolism and toxicity studies but will also enable the study of drug effects across a broad spectrum of hepatocytes lines that might contain the genetic variations of the human population. For example, P450 polymorphism is the main cause of metabolism and toxicity variation of drugs in humans. Hepatocytes derived from iPSCs carrying these polymorphisms would be of great value for early drug safety evaluation. Although several inherited hepatic diseases have been successfully modeled *in vitro* by hepatocytes derived from patient-specific iPSCs^[53, 54], the current differentiation protocols are still lacking in terms of generating highly pure and phenotypic stable hepatocytes. The use of three-dimensional culture and co-culture systems to mimic the *in vivo* hepatic context are among the strategies used to enhance the generation of hepatocytes^[49]. Given the continuous effort devoted to improve the differentiation methods, the future of applying iPSC-derived hepatocytes in drug screening and safety evaluation is promising.

Small molecules promoting iPSC generation

Although iPSCs have enormous potential, the clinical application of iPSC technology is still hindered by safety concerns and relatively low efficiency. In addition, the exact mechanisms underlying somatic cell reprogramming also remain to be elucidated. Considerable effort has been expended to modify the original reprogramming protocol, including using reduced number of factors^[9], non-integrating gene delivery approaches^[10], or cell permeable proteins to trigger the reprogramming^[11], to improve safety and make the iPSCs more amendable for clinical use. Compared to the aforementioned methods, small-molecule drugs have several unique advantages, such as structural versatility and being easy to control in a time- and concentration-dependent way. Many small molecules have been found to be extremely useful in steering reprogramming toward a faster, more efficient, and directed process (Table 2).

Epigenetic modifiers

Epigenetic mechanisms preside over our genetic information and enable the normal development of all cells and tissues in our body. The reprogramming process demonstrates the profound flexibility of the mammalian epigenome, indicating modulators of epigenome are critically involved in iPSC generation^[55]. Accordingly, small molecules that directly modulate the epigenetic enzymes or mechanisms, which in turn change DNA or histone modifications, have been demonstrated to affect reprogramming. Histone deacetylase inhibitors (HDACi) and DNA methyltransferase inhibitors are among the earliest discovered compounds that facilitate the generation of iPSCs^[56].

In particular, one study demonstrated that VPA, an HDACi,

improves 4-factor-mediated reprogramming efficiency by more than 100-fold^[56]. VPA, vorinostat (suberoylanilide hydroxamic acid, SAHA), and Trichostatin A (TSA) also improve the induction efficiency of mouse and human iPSCs with reduced numbers of factors (Oct4 and Sox2)^[56, 57]. Furthermore, VPA allowed successful generation of iPSCs with only recombinant proteins and without any genetic materials^[11]. BIX-01294 (BIX), a small-molecule inhibitor of histone methyltransferase (HMTase), G9a, has been demonstrated to significantly enhance the reprogramming efficiency and replace some of the transcription factors required for reprogramming^[58]. When BIX is combined with BayK8644, an L-calcium channel agonist, a synergistic increase in the number and size of iPSC colonies is observed. Other histone modification enzymes, such as LSD1 (a H3K4 demethylase), are also involved in reprogramming. Small molecules targeting LSD1, including parnate and LiCl, have also been reported to facilitate iPSC induction^[59, 60]. In addition, Jhdm1a/1b, two known vitamin-C-dependent H3K36 demethylases, have been found to critically regulate vitamin C-enhanced reprogramming^[61].

DNA methylation is another common epigenetic mechanism involved in the expression regulation of genes. A DNA methyltransferase (DNMT) inhibitor, 5-azacytidine (5-aza), has been found to promote overall efficiency in the 4-factor-induced reprogramming of MEFs^[56]. RG108, another DNMT inhibitor, enhanced the reprogramming efficiency of MEFs transduced with Oct4 and Klf4 in combination with BIX^[58]. Recently, stress mediated-p38 activation has also been shown to facilitate iPSCs generation by reducing the global DNA methylation level^[62]. It is also interesting to note that epigenetic modifiers not only improve the efficiency of reprogramming but also improve the quality of the resulting iPSCs. TSA and 5-aza have been shown to erase the epigenetic memory of somatic cells in early passage iPSCs^[63, 64]. TSA has also been used to activate the expression of the imprinted *Dlk1-Dio3* locus^[65, 66], which is critical to the pluripotency of iPSCs.

MET/EMT modulators

The mesenchymal to epithelial transition (MET) is critically involved in somatic cell reprogramming. The mesenchymal-type fibroblasts undergo dramatic morphological changes to form tightly packed iPSC clones with distinct cell-cell interaction and gene expression profiles^[67]. Transforming growth factor β (TGF β) is a prototypical cytokine involved in the induction of epithelial to mesenchymal transition (EMT), the opposite process of MET, which plays important roles in stem cell differentiation and tumor metastasis. Recent studies have demonstrated the importance of the inhibition of TGF β signaling in the generation of mouse and human iPSCs. A-83-01, a small-molecule inhibitor of TGF β receptor I kinase/activin-like kinase 5 (ALK5), has been shown to be critical for the generation and maintenance of rat iPSCs in combination with the MEK and GSK-3 β inhibitors^[68]. Inhibiting the TGF β pathway with other ALK5 inhibitors, including SB-431542 and E-616452 (RepSox), enables faster and more efficient induction of iPSCs and bypasses the requirement for exogenous c-Myc

Table 2. Summary of small molecule compounds enhancing iPSC generation.

| Name | Targets | Effects | Ref |
|--|---|---|-----------|
| Chemicals promoting reprogramming | | | |
| Epigenetic modifiers | | | |
| Valproic acid | HDAC inhibitor | Promote KOS-induced reprogramming, enable the reprogramming of human fibroblasts transduced by OS | [56, 57] |
| TSA | HDAC inhibitor | Promote OSKM-induced reprogramming | [56] |
| SAHA | HDAC inhibitor | Promote OSKM-induced reprogramming | [56] |
| RG108 | DNMT inhibitor | In combination with BIX to improve KO-induced MEF reprogramming | [58] |
| 5-AZA | DNMT inhibitor | Promote OSKM-induced reprogramming | [56] |
| BIX-01294 | HMTase G9a inhibitor | Promote reprogramming of NPCs and MEFs transduced by OK | [58, 102] |
| Parnate | LSD1 inhibitor | Enable the reprogramming of human keratinocytes transduced by OK | [59] |
| LiCl | Inhibitor of LSD1 and GSK3 | Promote MEF reprogramming by OSKM | [60] |
| Vitamin C | Vitamin-C-dependent H3K36 demethylase (Jhdm1a/1b), antioxidant, p53, senescence | Enhance iPSC generation from both mouse and human somatic cells | [91] |
| Anisomycin and TNF- α | Activation of p38, reducing the global DNA methylation | Promote MEF reprogramming by OSKM | [62] |
| EMT/MET modulators | | | |
| SB-431542 | ALK4/5/7 inhibitor | Replace c-myc or Sox2 to promote MEF reprogramming | [69] |
| E-616452 (RepSox) | ALK5 inhibitor | Promote reprogramming, replace Sox2 in reprogramming | [70] |
| A-83-01 | ALK5 inhibitor | Combined with MEK and Rock inhibitors to improve human fibroblast reprogramming; support rat iPSC long-term self-renewal when combined with CHIR99021 and PDO325901 | [68, 103] |
| Thiazovivin | ROCK inhibitor | Increase reprogramming efficiency and accelerate reprogramming kinetics of human fibroblasts in conjunction with MEK and TGF inhibitors | [103] |
| Metabolic regulator (glycolysis pathway) | | | |
| PS48 | Activator of PDK1/PI3K/Akt | Promote human iPSC induction by Oct4 alone | [75] |
| Fructose 6-phosphate | Stimulate glycolytic metabolism | Promote human iPSC induction by Oct4 alone | [75] |
| Fructose 2,6-bisphosphate | Stimulate glycolytic metabolism | Promote human iPSC induction by Oct4 alone | [75] |
| Nicotinic acid | Stimulate glycolytic metabolism | Promote human iPSC induction by Oct4 alone | [75] |
| 2-Hydroxyglutaric acid (2-HA) | Stimulate glycolytic metabolism | Promote human iPSC induction by Oct4 alone | [75] |
| Quercetin | HIF pathway activation | Promote human iPSC induction by Oct4 alone | [75] |
| N-oxaloylglycine (NOG) | HIF pathway activation | Promote human iPSC induction by Oct4 alone | [75] |
| 2,4-dinitrophenol (DNP) | Uncouple the mitochondrial respiratory chain | Promote human iPSC induction by Oct4 alone | [75] |
| Other chemicals promoting reprogramming | | | |
| (+)Bayk 8644 | L-type Ca ²⁺ channel | Promote MEF reprogramming in combination with G9a inhibition | [58] |
| CD437 | RAR agonist | Promote OSKM-induced reprogramming | [92] |
| AM580 | RAR agonist | Promote OSKM-induced reprogramming | [92] |
| Dexamethasone | Steroid glucocorticoid | Promote OSKM-induced reprogramming | [56] |
| Rapamycin | Inhibitor of mTOR | Promote OSKM-induced reprogramming | [94] |
| PP242 | Inhibitor of mTOR | Promote OSKM-induced reprogramming | [94] |
| Resveratrol | Sirtuin activator | Promote OSKM-induced reprogramming | [94] |
| Fisetin | Sirtuin activator | Promote OSKM-induced reprogramming | [94] |
| Spermidine | An autophagy inducer | Promote OSKM-induced reprogramming | [94] |
| LY294002 | PI3K inhibitor | Promote OSKM-induced reprogramming | [94] |
| Curcumin | An antioxidant | Promote OSKM-induced reprogramming | [94] |
| Chemicals facilitating self-renewal of stem cells | | | |
| PDO325901+CHIR99021 (2i) | Inhibitor of MEK and GSK3 respectively | Eliminate differentiation of stem cells | [87] |
| 6-Bromoindirubin-3'-oxime (BIO) | GSK-3 inhibitor IX | Maintain human and mouse ESCs in an undifferentiated state without feeder cells | [88] |
| Pluripotin/SC1 | RasGAP and ERK1 | Support mESC self-renewal | [86] |
| Y-27632 | ROCK inhibitor | Enhance survival rate after cryopreservation and cloning efficiency of human ES and iPSCs | [104] |

iPSC, induced pluripotent stem cell; K, Klf4; O, Oct4; M, c-Myc; S, Sox2.

or Sox2^[69,70].

The Wnt/ β -catenin pathway also plays important roles in EMT/MET induction. The activation of Wnt leads to GSK-3 β inhibition and thus the stabilization of β -catenin. The activation of Wnt signaling by Wnt3a protein promotes iPSC generation from 3-factor (OSK) transduced MEFs^[71]. The GSK-3 β inhibitor CHIR99021, which strongly activates the β -catenin pathway, has been shown to be particularly useful in enhancing generation of mouse and human iPSCs without exogenous Sox2 and c-Myc^[59]. CHIR99021 is also one of the components of the famous 2i (PD0325901 and CHIR99021), which is extremely useful not only in maintaining ESCs but also in facilitating the final transition of certain partially reprogrammed cells into real iPSCs^[72]. Similarly, kenpaullone, a less specific GSK-3 β inhibitor that also inhibits cyclin-dependent kinase (CDK) and other kinases, has been found to be capable of replacing exogenous Klf4 to reprogram MEFs transduced by OSM^[73].

Metabolic regulators

In most differentiated cells, energy is produced mainly by mitochondrial oxidative phosphorylation. In contrast, stem cells mainly rely on anaerobic glycolysis followed by lactic acid fermentation in the cytosol to produce energy and thus fewer reactive oxygen species are generated^[74]. Consequently, the reprogramming of somatic cells that utilize mitochondrial oxidation to iPSCs would require a switch in metabolism. Therefore, small molecules modulating this process may enhance somatic cell reprogramming. PS48, an activator of 3-phosphoinositide-dependent protein kinase 1 (PDK1), significantly induces the expression of glycolytic genes via activation of PI3K/Akt pathway and facilitates human iPSC generation with one transcription factor Oct4^[75]. Although ESCs and iPSCs contain functional respiratory complexes that are able to consume oxygen, uncoupling protein 2 in these cells plays a critical role by preventing mitochondrial glucose oxidation and facilitating glycolysis^[76]. Thus, small molecules that uncouple the mitochondrial respiratory chain may also promote reprogramming to iPSCs. 2,4-dinitro-phenol (DNP), a well-known uncoupler, significantly increases reprogramming efficiency^[75]. The activation of the HIF pathway by N-oxalylglycine and quercetin also enhances reprogramming by promoting glycolytic metabolism. In contrast, compounds such as oxalate that block glycolytic metabolism inhibit reprogramming^[75]. These studies reveal that metabolism switching is another fundamental mechanism in somatic cell reprogramming that can be used as a promising target for the screening of iPSC-promoting molecules.

Small molecules promoting self-renewal of ESCs/iPSCs

The successful generation of iPSCs can be roughly divided into two steps: the transition (reprogram) from somatic cells to iPSCs and the propagation/self-renewal of iPSCs. Therefore, it is easy to understand that the chemicals used to promote ESC self-renewal should also promote the generation of iPSCs. The establishment and maintenance of early mouse ESCs

relied on feeder cells and serum^[77,78]. In the effort to identify more defined conditions for mESC culture, several growth factors were identified as being critical for mESC self-renewal. LIF (leukemia inhibitory factor) produced by the feeder cells and BMP4 (bone morphogenetic protein 4), a key factor existing in the serum, were found to be essential for maintaining the proliferation potential of mESCs^[79,80]. The maintenance of self-renewal can be viewed as a combination of continued proliferation and inhibition of differentiation through cross-talks among positive and negative regulators. LIF activates STAT3 to stimulate mESC proliferation, and BMP4 functions through SMAD to activate the inhibitors of differentiation. However, for human ESCs, other exogenous growth factors than LIF and BMP4 are required for their maintenance. For example, bFGF^[81,82], TGF- β /activin A^[83,84], Wnt^[81] proteins and insulin-like growth factor^[85] are reported to support the self-renewal of hESCs.

In addition to the growth factors, small molecules that can sustain the self-renewal of mESCs have been identified in more chemically defined conditions without feeder-, serum- and LIF supplementation. One example is pluripotin (also known as SC1)^[86], which does not directly act on the known pathways involved in ESC self-renewal, including LIF/STAT3, BMP/SMAD/ID, and Wnt/ β -catenin pathways, but rather through the inhibition of RasGAP and extracellular-signal-regulated kinase 1 (ERK1), two proteins with differentiation-inducing activity. Smith and colleagues^[87] demonstrated that using a chemical combination (2i) of PD0325901, an ERK1/2 inhibitor, to eliminate differentiation-inducing signaling from mitogen-activated protein kinase (MAPK), and CHIR99021, a GSK-3 β inhibitor to prevent MET, allows mESCs to maintain their innate program for self-replication, which does not require extrinsic instruction to maintain self-renewal^[88]. Many of these growth factors and compounds have also been applied to the induction of human or mouse iPSCs. Compared with conventional culture conditions, chemically defined medium can eliminate the highly variable factors contributed by feeder cells and the undefined composition of serum products and thus benefit the maintenance of large-scale, consistent and robust long-term ESC/iPSC cultures as well as derivation of new cell lines from difficult strains or species.

Other chemicals used in iPSC induction

Other signal pathways involved in cell stress, senescence and DNA damage have also been demonstrated to play a role in iPSC induction. Cellular senescence is considered a barrier to reprogramming, and the expression of reprogramming factors somehow triggers senescence by upregulating p53, p16^{INK4a}, and p21^{CIP1}, which contribute to the extremely low efficiency and slow kinetics of reprogramming^[89]. Blocking the p53 pathway greatly facilitates the generation of iPSCs^[90]. Vitamin C improves the reprogramming efficiency at least partially by reducing the senescence triggered by the p53 pathway^[91].

Recently, new evidence has indicated the roles of nuclear receptors in reprogramming. Dexamethasone, a glucocorticoid steroid, can promote OSKM-induced iPSC generation

in combination with 5-aza^[56]. It has been shown that ectopic expression of RAR α/γ and Nr5a2 greatly enhances reprogramming efficiency and kinetics, and both RAR α agonist CD437 and RAR γ agonist AM580 significantly enhance OSKM-induced reprogramming. It has been proposed that RAR ligands bind to RAR and promote the formation of RAR/RXR heterodimers, which then bind to the *Oct4* locus to activate and stabilize Oct4 expression^[92].

The mTOR pathway functions in many cellular processes, including cell growth, proliferation, differentiation, and survival. Recent studies suggest that the precise regulation of the mTOR activity plays a critical role in the successful reprogramming of somatic cells to form iPSCs^[93], and the inhibition of the mTOR pathway with rapamycin or PP242 enhances the efficiency of reprogramming^[94]. Interestingly, in addition to rapamycin, other longevity-promoting compounds, including two sirtuin activators (resveratrol and fisetin), an autophagy inducer (spermidine), a PI3K (phosphoinositide 3-kinase) inhibitor (LY294002) and an antioxidant (curcumin) have also been reported to promote somatic cell reprogramming to different extents.

Perspective

Currently, target-based drug screening is still the main approach employed in new drug identification and development. Though effective in certain occasions, the overall low success rate and high development cost indicate a requirement for better approaches for identifying new therapeutic

agents. Cell-based phenotypic assays represent a more physiological system and may provide better ways for drug screening and lead optimization, especially for complex diseases without a known molecular target. The development of the iPSC technology makes this approach more attractive because patient-specific iPSCs and subsequent disease-harboring tissue cells can be easily obtained for large-scale drug screening. Meanwhile, cardiomyocytes and hepatocytes derived from normal human iPSCs can be used for early toxicity evaluation, providing a more accurate and cost-effective system than many animal models. To meet these needs, the iPSC-based drug discovery platform will have to achieve safer and more efficient reprogramming, more robust directed differentiation and disease phenotyping. We summarized here that small molecule drugs can be used to improve the generation of iPSCs. Hopefully, the interplay between iPSC technology and small molecule compounds will push forward the applications of iPSC-based therapy and screening systems in the real world and eventually facilitate the development of new ways to treat diseases (Figure 1).

Acknowledgements

This work was supported by grants from the Chinese Academy of Sciences (XDA01040301) Ministry of Science and Technology of China (2009CB940900, 2010CB944900, 2011CB965104, 2011DFB30010) and the Shanghai Commission of Science and Technology (11DZ2292200).

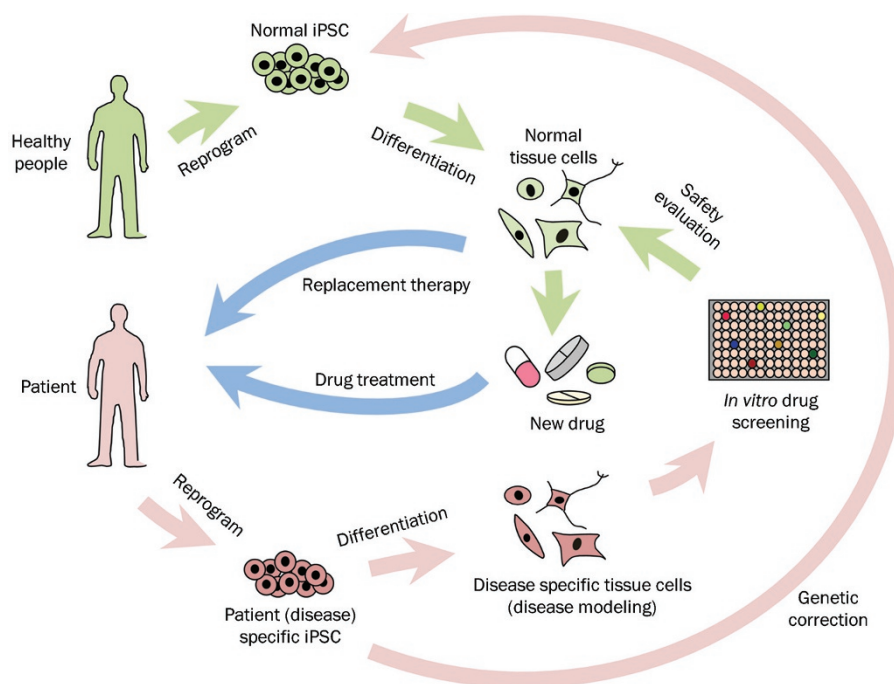


Figure 1. The use of human iPSCs in replacement therapy, disease modeling and drug development. iPSCs from healthy people can be differentiated into various normal tissue cells, which can be transferred into patients to replace the damaged cells or tissues or used for the safety evaluation of new drugs. iPSCs carrying disease-linked mutations can be derived from patients. These patient-specific iPSCs can be genetically corrected *in vitro* and used for autologous cell replacement therapy, or the iPSCs can be differentiated into tissue cells harboring the disease phenotype. These cells can be used to study the mechanisms of the disease and to screen new drug.

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