stem cell research&therapy

REVIEW

Induced pluripotent stem cell technology for disease modeling and drug screening with emphasis on lysosomal storage diseases

Hsiang-Po Huang[1], Ching-Yu Chuang[2] and Hung-Chih Kuo*[2,3]

Abstract

The recent derivation of disease-specific induced pluripotent stem cells (iPSCs) from somatic cells of patients with familial and sporadic forms of diseases and the demonstration of their ability to give rise to disease-relevant cell types provide an excellent opportunity to gain further insights into the mechanisms responsible for the pathophysiology of these diseases and develop novel therapeutic drugs. Here, we review the recent advances in iPSC technology for modeling of various lysosomal storage diseases (LSDs) and discuss possible strategies through which LSD-iPSCs can be exploited to identify novel drugs and improve future clinical treatment of LSDs.

Introduction

By using a combination of transcription factors that had previously yielded success in reprogramming mouse somatic cells [1], Yamanaka and colleagues demonstrated that human somatic cells can be directly reprogrammed to a pluripotent state similar to that of their embryonic stem cell (ESC) counterparts *in vitro* [2]. The human induced pluripotent stem cells (iPSCs) created by the reprogramming process exhibit the typical characteristics of human ESCs, showing morphology and growth requirements and surface and pluripotent-related marker expression similar to those of their inner cell mass-derived counterparts [2-4]. Furthermore, both *in vitro* differentiation and *in vivo* teratoma formation analyses indicate that human iPSCs are truly PSCs as they are able to give rise to cell types representing all three embryonic germ layers [2,5].

*Correspondence: kuohuch@gate.sinica.edu.tw
[2]Genomics Research Center, Academia Sinica, No. 128, Sec. 2, Academia Road, Nankang, Taipei 115, Taiwan
Full list of author information is available at the end of the article

Lysosomal storage diseases (LSDs) are individually rare but collectively common; their estimated total prevalence is 1 out of 8,000 live births [6]. As current therapies have limited effect and most LSDs progress relentlessly, therapies that are more efficient are urgently needed. To develop efficient therapies, a more thorough understanding of the pathophysiological development of LSDs at the cellular level is essential. Several LSD disease models have been established in knockout mice for disease modeling and drug tests [7-12]. However, at present, these models are not able to mimic the whole spectrum of LSD conditions. Therefore, lack of appropriate human cells affected by LSDs for drug screening and toxicity testing may be a major obstacle in the development of new therapies for LSDs. Since pluripotency reprogramming technology offers an easy and efficient means to generate patient-specific iPSCs, the iPSCs derived from patients with familiar or sporatic disease offer a valuable methodology through which to study the mechanisms involved in the initiation and progression of LSDs *in vitro* and further screen remedies for clinical treatment.

This review focuses on the current status of the application of iPSCs in LSD research by summarizing the LSD-iPSC cellular/ultrastructural findings that have been reported in the literature and by presenting useful strategies for drug designing/screening using LSD-iPSCs. We hope this review will encourage additional translational research in LSD drug development using this novel stem cell technology.

Lysosomal storage diseases

Lysosomes are unique intracellular organelles that contain an array of enzymes that are responsible for degrading aged organelles and disused macromolecules, such as glycoproteins, glycosaminoglycans (GAGs), and glycolipids. Lysosomes are involved in many important cellular functions, including autophagy, exocytosis, receptor recycling, phagocytosis, and membrane trafficking [13,14]. LSDs are a large and heterogeneous group of genetic disorders caused mainly by deficiencies in different kinds of lysosomal enzymes, which result in various

organelle dysfunctions relating to the accumulation of specific substrates (Table 1) [14]. LSDs also encompass two other types of rare disorders caused by either transport defects through the lysosomal membrane [14,15] or defective vesicular trafficking (Table 1) [14,16,17]. The majority of LSDs are clinically progressive and currently have no definite cures. Recent development of enzyme replacement therapy (ERT) has successfully alleviated the symptoms of patients with certain types of LSDs, such as Gaucher's disease, Pompe disease, Fabry disease, and type I, II, and VI mucopolysaccharidosis (MPS) [18], but the benefit of ERT on the neurological manifestations of LSDs is less obvious [19]. Hematopoietic stem cell transplantation [20], substrate reduction therapy [21], and pharmaceutical chaperones [22] have also been developed to treat selected patients with LSDs and showed beneficial effects [23]. Nevertheless, for patients with advanced-stage or late-onset LSDs, the current treatment results are still not satisfactory.

History and progress of induced pluripotent stem cell technology

In 2006, Yamanaka and colleagues [1,2] demonstrated that forced expression of only four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) is sufficient to convert both mouse and human fibroblasts into ESC-like cells. Other researchers reported similar results at almost the same time [24-26]. The cells generated were named iPSCs. Such a breakthrough circumvented the thorny ethical issues surrounding research that employs human embryos and also offered the hope of providing replaceable human cells with less immune rejection for use in clinical applications. A major disadvantage of iPSC technology is its low efficiency. However, an increasing number of modified protocols employing chemicals and small molecules have been forwarded to improve the efficiency of iPSC derivation; these methods have been reviewed extensively elsewhere [27-29]. In addition, alternative strategies have been developed to avoid the problem of integration of viral genes, including *piggyBac* vectors [30], recombinant proteins [31], modified mRNAs [32], microRNAs [33], and Sendai virus [34]. In general, however, it is accepted that the most efficient method to generate human iPSCs is still through lentiviral and retroviral transduction [35]. While iPSC research *in vitro* has progressed considerably, the large-scale application of iPSCs in clinical practice in the near future still hangs in the balance, mainly because of the concern of tumorigenicity that is comparable to that of ESCs. In addition, a recent report [36] demonstrated that the teratomas from inbred C57BL/6 fibroblast-derived iPSCs still cannot prevent the occurrence of immune rejection when transplanted back to the syngeneic mice, although Okita and colleagues [37] argued against this conclusion and reasoned that teratoma formation might not be a good approach through which to evaluate immune rejection. Consistent with this viewpoint, transplantations of mutation-corrected iPSC-derived cells into affected mice have been shown to result in the rescue of disease phenotypes in mice with sickle cell anemia [38]. Nevertheless, it should be borne in mind that *de novo* immunogenicity might be produced during iPSC derivation and maintenance, which could be caused by, for example, the viral antigens generated by viral vectors or the animal antigens contained in the serum or supplements used for cell culture. Despite all of these uncertainties regarding clinical application, it is well recognized that human iPSCs are an unprecedented and powerful tool that is highly promising for modeling numerous human genetic diseases *in vitro*.

Induced pluripotent stem cells for disease modeling and drug testing

So far, more than 40 iPSC disease models have been successfully generated from patients with genetic diseases [5], and the length of the list keeps increasing. Notable examples of models developed so far are Duchenne and Becker muscular dystrophy [7], Huntington disease [7], Shwachman-Diamond syndrome [7], Lesch-Nyhan syndrome [7], amyotrophic lateral sclerosis [39], spinal muscular atrophy [40], familial dysautonomia [41], dyskeratosis congenital [42], Friedreich's ataxia [43], fragile X syndrome [44], LEOPARD (lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retarded growth, deafness) syndrome [45], long-QT syndrome [46,47], Rett syndrome [48], and Hutchinson-Gilford progeria [49], although not all of these iPSCs exhibit disease-specific phenotypes. The derivatives of some of these disease-specific iPSCs have been employed as in vitro disease models to test the phenotype-correcting effects of small numbers of promising drugs, such as neurons differentiated from spinal muscular atrophy-specific iPSCs [40] and Rett syndrome-specific iPSCs [48] and cardiomyocytes differentiated from iPSCs for long-QT syndrome [47], suggesting the probability of their use as platforms for performing high-throughput screenings of large chemical libraries to identify novel drug candidates for these diseases.

Lysosomal storage disease-specific iPSCs and their cellular pathology

Gaucher disease: the initial proof-of-principle lysosomal storage disease-iPSCs

Since there is no cure for most LSDs in current medicine, LSD-specific iPSCs may provide a unique opportunity for dissecting unexplored disease pathogeneses and identifying new drugs. Several disease-specific iPSCs have

Table 1. Clinical profiles of common lysosomal storage diseases

Disease	Gene/defective protein	Function	Clinical symptoms (*in vivo* phenotypes)	Biochemical/cellular pathology	Available therapies[a]
Pompe disease	*GAA*/acid alpha-glucosidase	Degrading glycogen in cellular vacuoles	Cardiac and respiratory failure; muscle weakness	Glycogen storage; autophagic build-up	Myozyme; Lumizyme; DNJ (trial)
Sphingolipidoses					
Fabry disease	*GLA*/alpha-galactosidase A	Hydrolyzing the terminal alpha-galactosyl moieties from glycolipids and glycoproteins	Cardiovascular problems; renal failure; pain and paresthesia in the extremities	Increase levels of GB3; laminated alternate electron-dense and lucent layers (Zebra bodies) in the cytoplasm	Fabrazyme; Replagal; DGJ (trial)
Gaucher disease	*GBA*/acid beta-glucocerebrosidase	Cleavage of the glucosylceramide to ceramide and glucose	Hepatosplenomegaly; pancytopenia; skeletal (type 1 and 3) or neurological problems (type 2 and 3)	Tubular structures in arrays inside dilated lysosomes under EM	Cerezyme; Zavesca (miglustat; NB-DNJ); isofagomine (trial)
Niemann-Pick disease, type A and B	*SMPD1*/acid sphingomyelinase	Converting sphingomyelin to ceramide	Hepatosplenomegaly; cherry-red maculae; pneumonia	Abnormal lipid profiles; lamellar inclusions under EM	rhASM (for type B, trial, not published)
Metachromatic leukodystrophy	*ARSA*/arylsufatase A	Hydrolyzing cerebroside sulfate to cerebroside and sulfate	Progressive neurological dysfunction; demyelination	Metachromatic deposits in the nervous system	BMT (may delay progress); rhARSA (for those who have received HSCT, trial, not published)
Krabbe disease (Globoid cell leukodystrophy)	*GALC*/beta-galactocerebrosidase	Hydrolyzing the galactose ester bonds of galactosylceramide	Diffuse neurological dysfunction; sensorimotor peripheral neuropathy	Accumulation of galactosylsphingosine in all tissues, filamentous and curvilinear inclusions	HSCT; BMT
Mucopolysaccharidoses (MPS): representative types					
Hurler syndrome (MPS IH)	*IDUA*/alpha-L-iduronidase	Hydrolyzes the terminal alpha-L-iduronic acid residues of the glycosaminoglycans	Hepatosplenomegaly; heart diseases; dysostosis multiplex; mental retardation	Accumulation of dermatan sulfate and heparan sulfate	HSCT; Aldurazyme
Hunter syndrome (MPS II)	*IDS*/iduronate sulfatase	Lysosomal degradation of heparan sulfate and dermatan sulfate	Growth deficiency; coarse face; stiff joints; mental retardation	Accumulation of dermatan sulfate and heparan sulfate	BMT; Elaprase
Sanfilippo syndrome B (MPS IIIB)	*NAGLU*/alpha-N-acetylglucosaminidase	Lysosomal degradation of heparan sulfate	Severe neurodegeneration; coarse face; mild organomegaly	Accumulation of heparan sulfate; abnormal Ca^{+2} homeostasis; Golgi abnormalities; abnormal neurite outgrowth	Miglustat (NB-DNJ, trail)
Sly syndrome (MPS VII)	*GUSB*/beta-glucuronidase	Lysosomal degradation of glucuronic acid-containing glycosaminoglycans	Heart disease; dysostosis multiplex; hepatosplenomegaly; hydrops fetalis; mental retardation	Progressive lysososmal storage in many tissues because of accumulation of dermatan sulfate and heparan sulfate	BMT

Continued overleaf

Table 1. Continued

Disease	Gene/defective protein	Function	Clinical symptoms (*in vivo* phenotypes)	Biochemical/cellular pathology	Available therapies[a]
Lipid storage disease					
Niemann-Pick disease, type C	*NPC1*/NPC1 protein	Regulating intracellular cholesterol trafficking	Hepatosplenomegaly; neurological deterioration	Low-cholesterol esterification; lamellar inclusions in foam cells	Zavesca (miglustat; NB-DNJ)
Ceroid lipofuschinosis, neuronal, type 1	*PPT1*/palmitoyl-protein thioesterase	Removing palmitate from cysteine residues during lysosomal degradation	Myoclonus; spasticity; ophthalmic problems; mental deterioration	Granular osmiophilic cytoplasmic deposits in cells under EM	Cystagon (cysteamine bitartrate) combined with N-acetylcysteine (trial); neural stem cells (trial)
Mucolipidosis					
I-cell disease	*GNPTAB*/N-Acetylglucosaminyl-phosphotransferase alpha and beta subunits	Catalyzing the synthesis of the M-6-P determinant on lysosomal hydrolases	Alveolar ridge hypertrophy; dysostosis multiplex; heart failure; joint limitation; thick tight skin; mental retardation	Increased lysosomal enzyme secretion; membrane-bound vacuoles containing electron-lucent or fibrillogranular contents	BMT
Oligosaccharidosis					
Fucosidosis	*FUCA1*/alpha-L-fucosidase	Degrading fucose-containing glycoproteins and glycolipids	Angiokeratoma; psychomotor retardation; coarse face; dysostosis multiplex	Increased oligosaccharide secretion; elevated sweat chloride; vacuolated lymphocytes	HSCT; BMT
Transport defects through the lysosomal membrane					
Cystinosis	*CTNS*/cystinosin	Transporting cystine out of lysosomes	Failure to thrive; renal tubular Fanconi syndrome; hepatosplenomegaly; skeletal problems	Characteristic crystals within lysosomes causing cell expansion	Cystagon (cysteamine bitartrate)
Defective vesicular traffic					
Chediak-Higashi syndrome	*CHS1*/LYST protein	Regulating protein trafficking to and from lysosomes	Recurrent pyogenic infections; ophthalmic problems; hepatosplenomegaly; neurological dysfunction	Giant granules in muscle cells; giant lysosomes; altered vesicular fusion; defective antigen presentation	BMT; HSCT

[a]Regardless of their efficacy, only drugs that have been approved or are undergoing clinical trials are listed. BMT, bone marrow transplantation; DGJ, 1-deoxygalactonojirimycin; DNJ, 1-deoxynojirimycin; EM, electron microscopy; GB3, globotriaosylceramide; HSCT, hematopoietic stem cell therapy; M-6-P, mannose-6-phosphate; NB-DNJ, N-butyl-1-deoxynojirimycin; rhARSA, recombinant arylsufatase A; rhASM, recombinant acid sphingomyelinase.

been successfully generated from either mouse models for LSDs or patients with LSDs (Table 2). The iPSCs derived from a patient with Gaucher disease type III accounted for perhaps the first reported human LSD-iPSC line established [7], although few Gaucher disease-specific phenotypes have been described in detail.

Mouse lysosomal storage disease-specific iPSCs

Using tail-tip fibroblasts from mouse models of Fabry disease [8], Krabbe disease [8], MPS VII [8], and Pompe disease [9], Eto and colleagues have generated corresponding disease-specific iPSCs and characterized them. In addition to reporting deficient enzyme activities and substrate accumulation in these cells, the authors reported impaired embryonic body formation in MPS VII-specific iPSCs [8], a novel phenotype that, as the authors suggested, is possibly attributed to an elevated level of hyaluronic acid and may not be easily identified without using disease-specific iPSCs. According to the authors, two *in vivo* phenotypes are possibly related to

Table 2. Disease modeling and drug testing of lysosomal storage disease induced pluripotent stem cells recorded in the literature

Disease	Species	Original cell type	Cell type of interest	Disease phenotype[a]	Drug testing	References
Pompe disease	Mouse	Fibroblasts	Skeletal muscle cells	Glycogen accumulation	No	[9]
Pompe disease	Human	Fibroblasts	Cardiomyocytes	Glycogen storage and abnormal morphology and functions of mitochondria	Yes	[10]
Fabry disease	Mouse	Fibroblasts	Cardiomyocytes	Gb3 accumulation	No	[8]
Krabbe disease	Mouse	Fibroblasts	Neural stem cells	Reduced beta-galactocerebrosidase activity	No	[8]
Gaucher disease (type III)	Human	Fibroblasts	ND	ND	No	[7]
Hurler syndrome (MPS 1H)	Human	Keratinocytes and bone marrow mesenchymal stem cells	Hematopoietic cells	Lysosomal storage of glycosaminoglycans	No	[11]
MPS IIIB	Human	Fibroblasts	Neural stem cells and neurons	Storage vesicles associated with disorganized Golgi complex	Yes	[12]
MPS VII	Mouse	Fibroblasts	Unknown	Elevated levels of hyaluronic acid and impaired formation of embryoid bodies	No	[8]

[a]Except for the impaired embryoid body formation found in mucopolysaccharidosis (MPS) VII induced pluripotent stem cells (iPSCs), all lysosomal storage disease iPSC phenotypes have been reported in patient tissues or cells. Gb3, globotriaosylceramide; ND, not described.

impaired embryoid body formation in MPS VII iPSCs: (1) the lower-than-expected 25% of MPS VII ($GUSB^{-/-}$) mice born from heterozygous mating based on Mendelian inheritance and (2) hydrops fetalis, which is known to be relatively common in patients with MPS VII [8]. Moreover, the same group has shown that mouse Pompe disease iPSCs can be differentiated into skeletal muscles that are positively stained for myosin heavy chain, accumulate glycogen in lysosomes, and have typical ultrastructural features, including Z-, I-, A-, and H-bands [9]. Such an achievement is remarkable because skeletal muscles are among the most difficult mammalian cell types to obtain from *in vitro* differentiation of pluripotent stem cells [50].

Human Pompe disease

Using a unique acid alpha-glucosidase (GAA) rescue-based strategy and fibroblasts from two patients with Pompe disease, we also successfully generated four Pompe disease-specific iPSC lines [10]. All of these lines exhibit Pompe disease-specific phenotypes, such as very low GAA activity and high glycogen content, and can be differentiated into cardiomyocytes that have disarrayed myofibrils and abundant glycogen-containing vacuoles. Unexpectedly, we also found that Pompe disease iPSCs have defective cellular respiration (whereas ESCs and normal iPSCs do not) and this is supported by our finding that the mitochondria of cardiomyocytes derived from Pompe disease iPSCs exhibit abnormal morphology. We have tested several drugs/chemicals by using Pompe disease iPSC-derived cardiomyocytes and found that glycogen accumulation can be reduced by recombinant GAA and that the deteriorated mitochondrial functions can be partially rescued by L-carnitine. In addition, using comparative transcriptome analysis, we have identified six marker genes whose expression robustly correlates with the therapeutic effect of recombinant GAA or L-carnitine [10]. We are currently using these Pompe disease iPSCs to test the therapeutic effects of other compounds (for example, autophagy inhibitors) because excessive autophagic build-ups have been documented as an important feature of cells of patients with Pompe disease [51,52].

Human mucopolysaccharidosis 1H

Presuming that iPSC-derived hematopoietic cells may have fewer immunological complications than regular donor cells in hematopoietic cell transplantation (HCT), Tolar and colleagues [11] generated iPSCs from a patient with MPS type IH, a severe form of alpha-L-iduronidase deficiency that can be treated by HCT but not by ERT, and successfully corrected the gene defect by using iPSCs transduced with lentivirus harboring the wild-type gene (called *IDUA*) encoding this enzyme. The authors reported that alpha-L-iduronidase activity is not required for stem cell renewal and that MPS IH-iPSCs already have lysosomal storage of GAG. Moreover, MPS IH-iPSCs can be differentiated into hematopoietic progeny

with a colony-forming capacity comparable to those of *IDUA*-corrected and wild-type iPSC-derived hematopoietic progeny. The authors emphasized the advantages of iPSCs for possible application in HCT; for example, iPSCs are more feasible for long-term culture, and gene correction of iPSCs is easier than that of hematopoietic stem cells, which are more sensitive to *ex vivo* manipulation. Clarification of whether such *IDUA*-corrected iPSCs can be used for therapeutic purposes without immunological complications awaits further studies.

Human mucopolysaccharidosis IIIB

A prominent advantage of iPSC technology is that it offers access to patient neuronal cells, because neuronal cells are not difficult to derive from iPSCs [40]. This is critical for studying diseases – such as MPS type IIIB, a fatal LSD caused by the deficiency of α-N-acetylglucosaminidase – that involve primarily the central nervous system. Lemonnier and colleagues [12] successfully generated MPS IIIB-iPSCs and demonstrated that severe cellular pathology, including storage vesicles and disorganized Golgi complex, exists in undifferentiated iPSCs and differentiated neurons but not in neuronal progenitors. It is worth noting that the authors used exogenous recombinant enzyme to complement the enzymatic defect in order to clone MPS IIIB-iPSCs efficiently, an approach similar to our rescue strategy mentioned above [10]. Considering quantitative reverse transcription-polymerase chain reaction and Western blot results, the authors suggested that accumulation of heparin sulfate modifies the extracellular matrix constituents and related signaling pathways, which cause disorganization of Golgi architecture. Mild phenotypes in floating neuronal progenitors were explained by their non-adherent nature and less dependence on extracellular matrix-bound signals.

Strategies for drug design and screening for lysosomal storage diseases based on iPSCs

1. Pharmacological chaperones

The introduction above reveals that researchers around the world have generated proof-of-principle patient-specific iPSCs for several LSDs. Most of the published results of LSD-iPSC research have not advanced to the stage of drug design or medium-scale drug testing, not to mention attempts at high-throughput screening of chemical libraries for novel drug targeting of LSDs using iPSCs. However, compared with other disease iPSCs, LSD-iPSCs are more suitable for the purpose of drug design and high-throughput chemical screening because they are caused by defects of lysosomal enzymes, whose activities are measurable *in vitro* on a large-scale basis and the accumulated lysosomal substrates in LSDs can be assayed by either biochemical or immunocytochemical methods. Moreover, novel drugs can be designed or identified to enhance the activity, stability, or trafficking of mutant enzymes by assisting their folding or to target the pathways that synthesize the accumulated substrates. One class of small molecules termed pharmacological chaperones, which are reversible and competitive inhibitors of their target enzymes, may be suitable drug candidates.

In the past decade, many pharmacological chaperones have been developed to target the affected enzymes in various LSDs; this topic has been extensively reviewed elsewhere [22,53]. The chaperones can be taken orally, cross the blood-brain barrier, and have biodistributions that are better than those of ERT. Some of these chaperones are already prescribed formally in clinical practice [53]. The common strategies for identifying candidate pharmacological chaperones include both looking for molecules that have structural homology with the target natural substrates [54] and direct high-throughput screenings of compound libraries [55]. The methodology comprises initial *in vitro* assays such as enzyme inhibition assays in different pH environments [22,56], physical stability assays [22,57] using recombinant wild-type enzymes, and subsequent cell-based assays to estimate the effect of chaperones on enzyme activity and enzyme trafficking [22]. Chaperones identified to work for wild-type enzymes are not necessarily helpful for mutant enzymes and need be tested in different patients' fibroblasts or cell lines to evaluate the therapeutic effect [22]. For this purpose, LSD-iPSCs can offer a limitless source of human diseased cells containing various mutations for the second-round drug screening that is based on various cell-based assays, especially for those biochemical or cellular phenotypes seen in distinct differentiated cells (for example, neurons) that can be obtained only through directed differentiation of iPSCs. It is also theoretically possible that LSD-specific iPSCs can be used directly in first-round or even large-scale cell-based screening using similar strategies because a number of these cell-based assays have been successfully developed to meet the requirements of high-throughput screening formats [22], including the high-content imaging platforms [58] to evaluate enzyme trafficking.

2. Proteostasis regulators and other compounds

In addition to pharmacological chaperones, other ways to improve the protein folding of mutant enzymes are available. Two alternative methods have been developed [59-61]. First, it has been demonstrated [59,60] that two common L-type calcium channel blockers [59], either diltiazem or verapamil, and ryanodine receptor blockers, such as lacidipine [60], can partially restore the activity of two glucocerebrosidase mutants in fibroblasts derived from patients with Gaucher disease; the authors suggested

that these drugs exert their effects by upregulating a subset of molecular chaperones, such as BiP and Hsp40, which in turn ameliorate the capacity of the endoplasmic reticulum to rescue misfolded mutant enzymes. Second, Mu and colleagues [61] showed that two proteostasis regulators, celastrol and MG-132, can increase the concentrations and functions of mutant enzymes associated with two LSDs: Gaucher disease and Tay-Sachs disease. Moreover, the authors demonstrated that the combined use of pharmacological chaperones and such proteostasis regulators can generate a synergistic rescue effect on mutant enzymes in cells derived from patients with either LSD [61]. A more comprehensive review on the multiple aspects of protein folding or degradation that are related to pharmacological intervention can be found elsewhere [62]. Obviously, future efforts should be aimed at identifying more compounds of these two classes by using LSD-iPSCs and proving that the chemicals identified by using these strategies can be successfully applied next in animal studies and finally in clinical trials.

On the other hand, novel adjunct therapies also deserve to be developed to better preserve various cellular functions after correcting the disease-specific cellular pathology in different organelles (for example, the autophagic build-up and mitochondrial dysfunction in Pompe disease [52] and Golgi abnormalities in MPS type IIIB [12]). In this respect, candidate drugs may include some well-known autophagy inhibitors used in clinical trials [63], drugs or nutrient supplements [64] for treating mitochondrial dysfunction, and chemicals known to reverse endoplasmic reticulum-to-Golgi trafficking defects [65]. High-throughput screening of chemical libraries for this purpose is also a possibility because similar approaches in other fields using ESCs/iPSCs have been reported [66,67] and because proper screening-based formats of some amenable biochemical and cellular assays for such organelle dysfunctions have been reported [65,68,69] and may be exploited in the iPSC system as well. However, this is still a challenging task because successful purification of differentiated cells is a prerequisite and iPSCs may need to be passaged as single cells that can survive. A Rho-associated kinase inhibitor [70] or Accutase (Millipore Corporation, Billerica, MA, USA) [71] may be helpful to achieve this goal.

Caveats in drug screening for lysosomal storage diseases based on iPSCs

Several caveats are associated with using patient-specific iPSCs for drug screening [72,73]. First, mutational heterogeneity exists in most LSDs, but establishment of patient-specific iPSCs is time-consuming and labor-intensive. Thus, it may not be practical for a single laboratory to generate patient-specific iPSCs for all of the mutations found in a single disease, and determining whether the drugs identified to work for certain mutants are also helpful for other mutants would be a problem. Second, as seen in other classic cell-based platforms for drug screening, there is no guarantee that drug candidates identified from *in vitro* assays can be used successfully *in vivo*. Third, to test drugs that target the phenotypes existing only in differentiated cells (for example, electrophysiological anomalies in cardiomyocytes), a highly homogenous cell population differentiated from iPSCs may be needed to obtain consistent readouts [22]. Therefore, extensive collaborations among different laboratories on the basis of consensus and standardized protocols for generating iPSC lines and comparing drug effects will be essential. Moreover, other assays and systems – such as using LSD animal models to determine the pharmacokinetics, pharmacodynamics, and toxicities of candidate drugs – should always be used to complement iPSC-based drug screening. Finally, a more efficient and economic, and less time-consuming, set of protocols for obtaining homogenous differentiated cell types of interest in LSDs should be established in advance.

Conclusions

iPSC technology offers a revolutionary method for modeling LSDs and other diseases and the hope of future cell-based therapy. Since most LSDs are characterized by defects in enzymes whose activities are readily measurable *in vitro*, disease-specific iPSCs offer an ideal *in vitro* cellular system for designing LSD-specific pharmacological chaperones and for possible high-throughput compound screening. However, the application of iPSC technology for drug discovery is still at an early stage, and several major challenges – such as the difficulty of generating highly enriched disease-relevant desired cell types in large quantities from diseased iPSCs, the influence of culture and reprogramming artifacts on cell behavior, and the inability to recapitulate disease features in the diseased iPSC derivatives – must be resolved before it can be rendered an efficient and robust system for developing drugs targeting LSDs. With the advances in generating transgenic human pluripotent stem cells [74], the difficulty of enriching desired cell types from differentiating diseased iPSCs is likely to be resolved soon as specific cell types can be purified from cell mixtures by lineage-specific genetic markers or directly differentiated by ectopically expressed lineage determinant(s) in diseased iPSCs or both. In addition to iPSCs, induced somatic cells, which directly convert from fibroblasts by defined transcription factors [75,76], can potentially provide another source of disease-relevant cell types for the purpose of disease modeling. The advantage of the direct cell fate conversion technology is that a desired cell type can be directly generated from a patient's fibroblasts

by using a fast and simple protocol without the need of further cell purification. However, it has been suggested that the conventional pathological phenotypes of certain forms of diseases depend on cell interaction and may require a longer time to arise in a disease iPSC model [77,78]. Nevertheless, the disease process might be initiated much earlier than the emergence of clinical symptoms. Therefore, iPSC disease modeling can potentially provide an opportunity for earlier identification of phenotypic changes in diseases of interest. Despite these concerns, evidence supporting iPSC disease modeling of genetic diseases as a valuable *in vitro* cellular system through which to understand the mechanisms underlining the pathologies of diseases and future drug discovery has rapidly accumulated in the past few years.

This article is part of a thematic series on *Stem cell research in the Asia-Pacific* edited by Oscar Lee, Songtao Shi, Yufang Shi and Ying Jin. Other articles in the series can be found online at http://stemcellres.com/series/asiapacific

Abbreviations

ERT, enzyme replacement therapy; ESC, embryonic stem cell; GAA, acid alpha-glucosidase; GAG, glycosaminoglycans; HCT, hematopoietic cell transplantation; iPSC, induced pluripotent stem cell; LSD, lysosomal storage disease; MPS, mucopolysaccharidosis.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Medical Research and Department of Pediatrics, National Taiwan University Hospital, No. 7, Chung Shan S. Rd., Zhongshan, Taipei 100, Taiwan. [2]Genomics Research Center, Academia Sinica, No. 128, Sec. 2, Academia Road, Nankang, Taipei 115, Taiwan. [3]Institute of Cellular and Organismic Biology, Academia Sinica, No. 128, Sec. 2, Academia Road, Nankang, Taipei 115, Taiwan.

Published: 28 August 2012

References

1. Takahashi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.** *Cell* 2006, **126:**663-676.
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S: **Induction of pluripotent stem cells from adult human fibroblasts by defined factors.** *Cell* 2007, **131:**861-872.
3. Hoffman LM, Carpenter MK: **Characterization and culture of human embryonic stem cells.** *Nat Biotechnol* 2005, **23:**699-708.
4. Chen HF, Chuang CY, Lee WC, Huang HP, Wu HC, Ho HN, Chen YJ, Kuo HC: **Surface marker epithelial cell adhesion molecule and E-cadherin facilitate the identification and selection of induced pluripotent stem cells.** *Stem Cell Rev* 2011, **7:**722-735.
5. Robinton DA, Daley GQ: **The promise of induced pluripotent stem cells in research and therapy.** *Nature* 2012, **481:**295-305.
6. Meikle PJ, Hopwood JJ, Clague AE, Carey WF: **Prevalence of lysosomal storage disorders.** *JAMA* 1999, **281:**249-254.
7. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ: **Disease-specific induced pluripotent stem cells.** *Cell* 2008, **134:**877-886.
8. Meng XL, Shen JS, Kawagoe S, Ohashi T, Brady RO, Eto Y: **Induced pluripotent stem cells derived from mouse models of lysosomal storage disorders.** *Proc Natl Acad Sci U S A* 2010, **107:**7886-7891.
9. Kawagoe S, Higuchi T, Meng XL, Shimada Y, Shimizu H, Hirayama R, Fukuda T, Chang H, Nakahata T, Fukada S, Ida H, Kobayashi H, Ohashi T, Eto Y: **Generation of induced pluripotent stem (iPS) cells derived from a murine model of Pompe disease and differentiation of Pompe-iPS cells into skeletal muscle cells.** *Mol Genet Metab* 2011, **104:**123-128.
10. Huang HP, Chen PH, Hwu WL, Chuang CY, Chien YH, Stone L, Chien CL, Li LT, Chiang SC, Chen HF, Ho HN, Chen CH, Kuo HC: **Human Pompe disease-induced pluripotent stem cells for pathogenesis modeling, drug testing and disease marker identification.** *Hum Mol Genet* 2011, **20:**4851-4864.
11. Tolar J, Park IH, Xia L, Lees CJ, Peacock B, Webber B, McElmurry RT, Eide CR, Orchard PJ, Kyba M, Osborn MJ, Lund TC, Wagner JE, Daley GQ, Blazar BR: **Hematopoietic differentiation of induced pluripotent stem cells from patients with mucopolysaccharidosis type I (Hurler syndrome).** *Blood* 2010, **117:**839-847.
12. Lemonnier T, Blanchard S, Toli D, Roy E, Bigou S, Froissart R, Rouvet I, Vitry S, Heard JM, Bohl D: **Modeling neuronal defects associated with a lysosomal disorder using patient-derived induced pluripotent stem cells.** *Hum Mol Genet* 2011, **20:**3653-3666.
13. Luzio JP, Pryor PR, Bright NA: **Lysosomes: fusion and function.** *Nat Rev Mol Cell Biol* 2007, **8:**622-632.
14. Parkinson-Lawrence EJ, Shandala T, Prodoehl M, Plew R, Borlace GN, Brooks DA: **Lysosomal storage disease: revealing lysosomal function and physiology.** *Physiology (Bethesda)* 2010, **25:**102-115.
15. Durand P: **Recent progress of lysosomal diseases.** *Enzyme* 1987, **38:**256-261.
16. Ward DM, Griffiths GM, Stinchcombe JC, Kaplan J: **Analysis of the lysosomal storage disease Chediak-Higashi syndrome.** *Traffic* 2000, **1:**816-822.
17. Feng L, Novak EK, Hartnell LM, Bonifacino JS, Collinson LM, Swank RT: **The Hermansky-Pudlak syndrome 1 (HPS1) and HPS2 genes independently contribute to the production and function of platelet dense granules, melanosomes, and lysosomes.** *Blood* 2002, **99:**1651-1658.
18. Lachmann RH: **Enzyme replacement therapy for lysosomal storage diseases.** *Curr Opin Pediatr* 2011, **23:**588-593.
19. Begley DJ, Pontikis CC, Scarpa M: **Lysosomal storage diseases and the blood-brain barrier.** *Curr Pharm Des* 2008, **14:**1566-1580.
20. Malatack JJ, Consolini DM, Bayever E: **The status of hematopoietic stem cell transplantation in lysosomal storage disease.** *Pediatr Neurol* 2003, **29:**391-403.
21. Ficicioglu C: **Review of miglustat for clinical management in Gaucher disease type 1.** *Ther Clin Risk Manag* 2008, **4:**425-431.
22. Valenzano KJ, Khanna R, Powe AC, Boyd R, Lee G, Flanagan JJ, Benjamin ER: **Identification and characterization of pharmacological chaperones to correct enzyme deficiencies in lysosomal storage disorders.** *Assay Drug Dev Technol* 2011, **9:**213-235.
23. Pineda M, Wraith JE, Mengel E, Sedel F, Hwu WL, Rohrbach M, Bembi B, Walterfang M, Korenke GC, Marquardt T, Luzy C, Giorgino R, Patterson MC: **Miglustat in patients with Niemann-Pick disease Type C (NP-C): a multicenter observational retrospective cohort study.** *Mol Genet Metab* 2009, **98:**243-249.
24. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA: **Induced pluripotent stem cell lines derived from human somatic cells.** *Science* 2007, **318:**1917-1920.
25. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R: ***In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state.** *Nature* 2007, **448:**318-324.
26. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ: **Reprogramming of human somatic cells to pluripotency with defined factors.** *Nature* 2008, **451:**141-146.
27. Feng B, Ng JH, Heng JC, Ng HH: **Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells.** *Cell Stem Cell* 2009, **4:**301-312.
28. Nakhaei-Rad S, Bahrami AR, Mirahmadi M, Matin MM: **New windows to enhance direct reprogramming of somatic cells towards induced pluripotent stem cells.** *Biochem Cell Biol* 2012, **90:**115-123.
29. Zhu S, Wei W, Ding S: **Chemical strategies for stem cell biology and regenerative medicine.** *Annu Rev Biomed Eng* 2011, **13:**73-90.
30. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R, Cowling R, Wang W, Liu P, Gertsenstein M, Kaji K, Sung HK, Nagy A: **piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells.** *Nature* 2009, **458:**766-770.
31. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS: **Generation of human induced pluripotent stem cells by**

direct delivery of reprogramming proteins. *Cell Stem Cell* 2009, **4:**472-476.
32. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM,Rossi DJ: **Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA.** *Cell Stem Cell* 2010, **7:**618-630.
33. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, Nishikawa S, Tanemura M, Mimori K, Tanaka F, Saito T, Nishimura J, Takemasa I, Mizushima T,Ikeda M, Yamamoto H, Sekimoto M, Doki Y, Mori M: **Reprogramming of mouse and human cells to pluripotency using mature microRNAs.** *Cell Stem Cell* 2011, **8:**633-638.
34. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M: **Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome.** *Proc Jpn Acad Ser B Phys Biol Sci* 2009, **85:**348-362.
35. Kiskinis E, Eggan K: **Progress toward the clinical application of patient-specific pluripotent stem cells.** *J Clin Invest* 2010, **120:**51-59.
36. Zhao T, Zhang ZN, Rong Z, Xu Y: **Immunogenicity of induced pluripotent stem cells.** *Nature* 2011, **474:**212-215.
37. Okita K, Nagata N, Yamanaka S: **Immunogenicity of induced pluripotent stem cells.** *Circ Res* 2011, **109:**720-721.
38. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R: **Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin.** *Science* 2007, **318:**1920-1923.
39. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K: **Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons.** *Science* 2008, **321:**1218-1221.
40. Ebert AD, Yu J, Rose FF Jr., Mattis VB, Lorson CL, Thomson JA, Svendsen CN: **Induced pluripotent stem cells from a spinal muscular atrophy patient.** *Nature* 2009, **457:**277-280.
41. Lee G, Papapetrou EP, Kim H, Chambers SM, Tomishima MJ, Fasano CA, Ganat YM, Menon J, Shimizu F, Viale A, Tabar V, Sadelain M, Studer L: **Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs.** *Nature* 2009, **461:**402-406.
42. Agarwal S, Loh YH, McLoughlin EM, Huang J, Park IH, Miller JD, Huo H, Okuka M, Dos Reis RM, Loewer S, Ng HH, Keefe DL, Goldman FD, Klingelhutz AJ, Liu L, Daley GQ: **Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients.** *Nature* 2010, **464:**292-296.
43. Ku S, Soragni E, Campau E, Thomas EA, Altun G, Laurent LC, Loring JF, Napierala M, Gottesfeld JM: **Friedreich's ataxia induced pluripotent stem cells model intergenerational GAATTC triplet repeat instability.** *Cell Stem Cell* 2010, **7:**631-637.
44. Urbach A, Bar-Nur O, Daley GQ, Benvenisty N: **Differential modeling of fragile X syndrome by human embryonic stem cells and induced pluripotent stem cells.** *Cell Stem Cell* 2010, **6:**407-411.
45. Carvajal-Vergara X, Sevilla A, D'Souza SL, Ang YS, Schaniel C, Lee DF, Yang L, Kaplan AD, Adler ED, Rozov R, Ge Y, Cohen N, Edelmann LJ, Chang B,Waghray A, Su J, Pardo S, Lichtenbelt KD, Tartaglia M, Gelb BD, Lemischka IR: **Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome.** *Nature* 2010, **465:**808-812.
46. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flügel L, Dorn T, Goedel A, Höhnke C, Hofmann F, Seyfarth M, Sinnecker D, Schömig A, Laugwitz KL: **Patient-specific induced pluripotent stem-cell models for long-QT syndrome.** *N Engl J Med* 2010, **363:**1397-1409.
47. Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, Boulos M, Gepstein L: **Modelling the long QT syndrome with induced pluripotent stem cells.** *Nature* 2011, **471:**225-229.
48. Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, Chen G, Gage FH, Muotri AR: **A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells.** *Cell* 2010, **143:**527-539.
49. Zhang J, Lian Q, Zhu G, Zhou F, Sui L, Tan C, Mutalif RA, Navasankari R, Zhang Y, Tse HF, Stewart CL, Colman A: **A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects.** *Cell Stem Cell* 2010, **8:**31-45.
50. Darabi R, Santos FN, Filareto A, Pan W, Koene R, Rudnicki MA, Kyba M, Perlingeiro RC: **Assessment of the myogenic stem cell compartment following transplantation of Pax3/Pax7-induced embryonic stem cell-derived progenitors.** *Stem Cells* 2011, **29:**777-790.
51. Fukuda T, Ahearn M, Roberts A, Mattaliano RJ, Zaal K, Ralston E, Plotz PH, Raben N: **Autophagy and mistargeting of therapeutic enzyme in skeletal muscle in Pompe disease.** *Mol Ther* 2006, **14:**831-839.
52. Raben N, Schreiner C, Baum R, Takikita S, Xu S, Xie T, Myerowitz R, Komatsu M, Van der Meulen JH, Nagaraju K, Ralston E, Plotz PH: **Suppression of autophagy permits successful enzyme replacement therapy in a lysosomal storage disorder--murine Pompe disease.** *Autophagy* 2010, **6:**1078-1089.
53. Parenti G: **Treating lysosomal storage diseases with pharmacological chaperones: from concept to clinics.** *EMBO Mol Med* 2009, **1:**268-279.
54. Fan JQ, Ishii S, Asano N, Suzuki Y: **Accelerated transport and maturation of lysosomal alpha-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor.** *Nat Med* 1999, **5:**112-115.
55. Maegawa GH, Tropak M, Buttner J, Stockley T, Kok F, Clarke JT, Mahuran DJ: **Pyrimethamine as a potential pharmacological chaperone for late-onset forms of GM2 gangliosidosis.** *J Biol Chem* 2007, **282:**9150-9161.
56. Flanagan JJ, Rossi B, Tang K, Wu X, Mascioli K, Donaudy F, Tuzzi MR, Fontana F, Cubellis MV, Porto C, Benjamin E, Lockhart DJ, Valenzano KJ, Andria G,Parenti G, Do HV: **The pharmacological chaperone 1-deoxynojirimycin increases the activity and lysosomal trafficking of multiple mutant forms of acid alpha-glucosidase.** *Hum Mutat* 2009, **30:**1683-1692.
57. Hamuro Y, Coales SJ, Southern MR, Nemeth-Cawley JF, Stranz DD, Griffin PR: **Rapid analysis of protein structure and dynamics by hydrogen/deuterium exchange mass spectrometry.** *J Biomol Tech* 2003, **14:**171-182.
58. Zanella F, Lorens JB, Link W: **High content screening: seeing is believing.** *Trends Biotechnol* 2010, **28:**237-245.
59. Mu TW, Fowler DM, Kelly JW: **Partial restoration of mutant enzyme homeostasis in three distinct lysosomal storage disease cell lines by altering calcium homeostasis.** *PLoS Biol* 2008, **6:**e26.
60. Wang F, Chou A, Segatori L: **Lacidipine remodels protein folding and Ca 2+ homeostasis in Gaucher's disease fibroblasts: a mechanism to rescue mutant glucocerebrosidase.** *Chem Biol* 2011, **18:**766-776.
61. Mu TW, Ong DS, Wang YJ, Balch WE, Yates JR 3rd, Segatori L, Kelly JW: **Chemical and biological approaches synergize to ameliorate protein-folding diseases.** *Cell* 2008, **134:**769-781.
62. Hartl FU, Bracher A, Hayer-Hartl M: **Molecular chaperones in protein folding and proteostasis.** *Nature* 2011, **475:**324-332.
63. Koul D, Shen R, Kim YW, Kondo Y, Lu Y, Bankson J, Ronen SM, Kirkpatrick DL, Powis G, Yung WK: **Cellular and *in vivo* activity of a novel PI3K inhibitor, PX-866, against human glioblastoma.** *Neuro Oncol* 2010, **12:**559-569.
64. Zammit VA, Ramsay RR, Bonomini M, Arduini A: **Carnitine, mitochondrial function and therapy.** *Adv Drug Deliv Rev* 2009, **61:**1353-1362.
65. Su LJ, Auluck PK, Outeiro TF, Yeger-Lotem E, Kritzer JA, Tardiff DF, Strathearn KE, Liu F, Cao S, Hamamichi S, Hill KJ, Caldwell KA, Bell GW, Fraenkel E,Cooper AA, Caldwell GA, McCaffery JM, Rochet JC, Lindquist S: **Compounds from an unbiased chemical screen reverse both ER-to-Golgi trafficking defects and mitochondrial dysfunction in Parkinson's disease models.** *Dis Model Mech* 2010, **3:**194-208.
66. Desbordes SC, Placantonakis DG, Ciro A, Socci ND, Lee G, Djaballah H, Studer L: **High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells.** *Cell Stem Cell* 2008, **2:**602-612.
67. Friedrichs S, Malan D, Matuscheck Y, Fleischmann BK, Sasse P: **High-throughtput screening of induced pluripotent stem cell-derived cardiomyocytes with long QT-syndrome 3.** *Acta Physiologica* 2012, **204 (Suppl 689):**189.
68. Huang SG: **Development of a high throughput screening assay for mitochondrial membrane potential in living cells.** *J Biomol Screen* 2002, **7:**383-389.
69. Li M, Chen X, Ye QZ, Vogt A, Yin XM: **A high-throughput FRET-based assay for determination of Atg4 activity.** *Autophagy* 2012, **8**.
70. Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T, Takahashi JB, Nishikawa S, Muguruma K, Sasai Y: **A ROCK inhibitor permits survival of dissociated human embryonic stem cells.** *Nat Biotechnol* 2007, **25:**681-686.
71. Bajpai R, Lesperance J, Kim M, Terskikh AV: **Efficient propagation of single cells Accutase-dissociated human embryonic stem cells.** *Mol Reprod Dev* 2008, **75:**818-827.
72. Ebert AD, Svendsen CN: **Human stem cells and drug screening: opportunities and challenges.** *Nat Rev Drug Discov* 2010, **9:**367-372.

73. Ebert AD, Liang P, Wu JC: **Induced pluripotent stem cells as a disease modeling and drug screening platform.** *J Cardiovasc Pharmacol* 2012, Jan 11. [Epub ahead of print].
74. Wang P, Rodriguez RT, Wang J, Ghodasara A, Kim SK: **Targeting SOX17 in human embryonic stem cells creates unique strategies for isolating and analyzing developing endoderm.** *Cell Stem Cell* 2011, **8:**335-346.
75. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M: **Direct conversion of fibroblasts to functional neurons by defined factors.** *Nature* 2010, **463:**1035-1041.
76. Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Bjorklund A, Lindvall O, Jakobsson J, Parmar M: **Direct conversion of human fibroblasts to dopaminergic neurons.** *Proc Natl Acad Sci U S A* 2011, **108:**10343-10348.
77. Schule B, Pera RA, Langston JW: **Can cellular models revolutionize drug discovery in Parkinson's disease?** *Biochim Biophys Acta* 2009, **1792:**1043-1051.
78. Lengerke C, Daley GQ: **Disease models from pluripotent stem cells.** *Ann N Y Acad Sci* 2009, **1176:**191-196.

doi:10.1186/scrt125
Cite this article as: Huang H-P, *et al*.: Induced pluripotent stem cell technology for disease modeling and drug screening with emphasis on lysosomal storage diseases. *Stem Cell Research & Therapy* 2012, **3**:34.