Preclinical Studies for Induced Pluripotent Stem Cell-based Therapeutics*

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Induced pluripotent stem cells (iPSCs) and their differentiated derivatives can potentially be applied to cell-based therapy for human diseases. The properties of iPSCs are being studied intensively both to understand the basic biology of pluripotency and cellular differentiation and to solve problems associated with therapeutic applications. Examples of specific preclinical applications summarized briefly in this minireview include the use of iPSCs to treat diseases of the liver, nervous system, eye, and heart and metabolic conditions such as diabetes. Early stage studies illustrate the potential of iPSC-derived cells and have identified several challenges that must be addressed before moving to clinical trials. These include rigorous quality control and efficient production of required cell populations, improvement of cell survival and engraftment, and development of technologies to monitor transplanted cell behavior for extended periods of time. Problems related to immune rejection, genetic instability, and tumorigenicity must be solved. Testing the efficacy of iPSC-based therapies requires further improvement of animal models precisely recapitulating human disease conditions.

The breakthrough discovery that specific sets of transcription factors can reprogram cell fate and generate induced pluripotent stem cells (iPSCs) from various cell types has opened many new possibilities for research on cell states, differentiation, pluripotency, and general cell identity but, most importantly, has catalyzed the development of a whole new field of regenerative medicine (1). The field is still in a relatively early stage regarding a clear understanding of underlying developmental processes, cell behavior, and biological effects after cell-grafting experiments. The use of iPSCs and their products for human applications poses many new challenges from the experimental and regulatory points of view due to the unique properties of the cells and novel mechanism of their action.

Testing iPSCs in Animal Disease Models

Reprogramming of somatic cells was originally demonstrated using mouse (2) and human (3) cells. The demonstration that the same transcription factors can reprogram non-human primate (4) and rat (5) cells indicates the conserved nature of mechanisms of inducing pluripotency among mammalian species. iPSCs were also obtained from rabbits (6), dogs (7), a variety of non-human primate species (8), and more recently, domestic ungulates, such as pig, cow, sheep, goat, and horse (reviewed in Ref. 9). A better understanding of the nature of the similarities and differences between human and animal stem cells and emulation of the behavioral, cellular, and molecular manifestations seen in human disease conditions in animal models should lead to interpretable testing of efficiency and should predict major complications and off-target effects of iPSC-based therapies.

Preclinical studies should be conducted using iPSC-derived products intended for clinical use. To prevent rejection of human cells in animal models, immunosuppressed or immuno-compromised animals should be considered. Humanized animal models, particularly mice, have reached some significant milestones, allowing reconstruction of human hematopoiesis and immunity. A variety of human disease conditions have been recapitulated in humanized mice, identifying mechanisms of relapse and suggesting novel therapeutic strategies (10). Future studies should increase the predictive capabilities of these models and facilitate the creation and use of humanized models based on large animal species (11), which can more reliably inform clinical trials.

For certain applications, human cells will not survive in the animal host, the immunosuppression protocol will not allow long-term observation, or immunomodulating drugs will affect the disease phenotype. Therefore, the use of autologous and homologous animal stem cell products, particularly in early stages of development of the intervention, might be considered. Immune reactions can significantly affect therapeutic efficiency and tumor formation. Because immune system reaction is a focus of another report in this thematic minireview series, we point out briefly that different mechanisms are predominantly acting on pluripotent and differentiated cells in syngeneic, allogeneic, and xenogeneic recipients (12). The recent finding that a mouse iPSC-induced response prevented teratoma formation in syngeneic transplantation was unexpected (13). Investigators from two other laboratories did not observe differences in the efficiency of transplantation and detected no immune response to terminally differentiated cells derived from syngeneic iPSCs or embryonic stem cells (14, 15). Explanations for these discrepancies might be genetic aberrations accumulated in iPSCs or heterogeneous populations of parental cells used in the original report. Further investigations will be required because immune rejection is one of the major concerns for iPSC-mediated replacement therapy.

Below are several examples of the use of iPSC-derived cells in animal disease models, highlighting that approaches to more precisely compare phenotypes and therapeutic outcomes among species should be developed (summarized in Table 1).

Liver Diseases—Successful strategies for efficient differentiation of human and animal iPSCs to hepatocytes have been...
developed (16). In many cases, these cells are very similar to primary hepatocytes, as judged by gene expression profiles, secreted proteins, and metabolism. These cells were engrafted into several animal models and were able to mature in vivo and perform normal functions in rodents. In some cases, the cells protected the animal from liver failure (17, 18). Significantly, a point mutation in the α₁-antitrypsin gene was corrected in human iPSCs, and derived liver cells showed normal cell func-

| Disease | Cell Type and Sources | Animal Disease Model | Cell Delivery | Major Results | Refs. |
|---------|----------------------|----------------------|---------------|---------------|------|
| Liver Diseases | Human hepatocyte-like cells; iPSCs from hepatocytes, bone marrow mesenchymal stem cells and liver fibroblasts | NOD/LtSCID/IL-2Rγ⁻/⁻ mice; liver cirrhosis | Intravenous injection | Engraftment rate 9-15%; human liver proteins in blood; 89% survival after transplantation | 17 |
| | Human hepatocyte-like cells from iPSC cell line (CFB46) | NOD-SCID mice; lethal fulminant hepatic failure | Intraspinal injection | Engraftment; 71% survival after transplantation | 18 |
| | Human hepatocyte-like cells; iPSCs from dermal fibroblasts with α₁-antitrypsin deficiency | Alb uPA⁺; Rag2⁻⁻ line | Intraspinal injection | Engraftment into mouse liver; functional restoration of A1AT in patient-derived cells | 19 |
| | Mouse retinal progenitor and photoreceptor precursor cells; iPSCs from dSRed dermal fibroblasts | Rho⁺ mouse (not forming functional rod receptors) | Subretinal injection | Cells integration; functional and morphological improvement | 29 |
| | Human retinal pigment epithelial cells; iPSCs from dermal fibroblasts | SCID mouse model of retinitis pigmentosa | Subretinal injection | Cell integration; functional improvement | 31 |
| | Swine rod photoreceptor cells; fetal fibroblast-derived iPSCs | Pigs lacking rod photoreceptors | Subretinal injection | Engraftment in the outer nuclear layer | 30 |
| Diabetes | Mouse β-cells; iPSCs from dermal fibroblasts | Type 1 and Type 2 diabetic mouse models | Intraperitoneal injection | Engraftment; improvement in diabetic phenotype | 41 |
| | Monkey pancreatic progenitors; iPSCs from dermal fibroblasts | NOD-SCID mice; induced diabetic model | Kidney capsule implantation | Improvement in diabetic phenotype | 42 |
| | Mouse β-cells; iPSCs from embryonic fibroblasts or pancreas epithelial cells | Type 1 diabetic NOD/SCID mice | Kidney capsule implantation | Improvement in diabetic phenotype | 45 |
| | Canine endothelial cells; iPSCs from adult adipose stromal cells and fibroblasts | SCID mice; myocardial infarction or hind limb ischemia | Intramyocardial or intramuscular injection | Cardiac or hind-limb engraftment; improved contractility, revascularization | 7 |
| Heart Disease | Human cardiomyocytes, endothelium and smooth muscle cells; iPSCs from dermal fibroblasts | RNU-RNU rats; myocardial infarction | Injection into the heart | Human cells engrafted and retained at 10 weeks; protection trend | 37 |
| | Porcine endothelial cells; iPSCs from adipose stromal cells | NOD/SCID mice; myocardial infarction | Intramyocardial injection | Short term engraftment; functional improvement | 38 |
| | Human endothelial cells; iPSCs from cord blood cells | Landrace pigs; myocardial infarction | Intramyocardial delivery | Cells detected up to 15 weeks after transplantation | 39 |
| | Human cardiomyocytes; iPSCs from dermal fibroblasts | Minipigs; induced cardiomyopathy | Transplantation. | Improved cardiac function; cells detected up to 8 weeks | 40 |
| Neurological Diseases | Rhesus monkey dopaminergic neural progenitors; iPSCs from dermal fibroblasts | Rhesus monkey; MPTP-induced Parkinson’s disease; autologous transplantation | Cells injected into caudate nucleus, putamen and substantia nigra | Engrafted cells were identified as neurons, astrocytes and oligodendrocytes | 20 |
| | Human neuroepithelial cells and dopaminergic neurons, iPSCs from fibroblasts | Rat model of Parkinson’s disease | Transplantation into striatum | Functional recovery; 25% of animals died due to tumors | 21 |
| | Human oligodendrocyte progenitors; iPSCs from fibroblasts | Rats; lyssolecithin-induced demyelinated optic chiasm | Transplantation into chiasm | Remyelination observed; cells developed into oligodendrocytes and integrated within the chiasm | 22 |
| | Human neurospheres; iPSCs from adult fibroblasts | NOD/SCID mouse spinal cord injury | Cells transplanted into lesion epicenter | Differentiated to mature brain cells; axonal regrowth and functional improvement | 23 |
| | Human neural stem/progenitor cells; iPSCs (2014) from adult dermal fibroblasts | Marmoset spinal cord injury | Cells injected into the lesion epicenter | Differentiated to mature brain cells; axonal regrowth and functional improvement | 24 |
| | Mouse iPSCs from embryonic fibroblasts (iPS-MEF-Ng-Ng20D-17) | C57BL/6N mouse stroke model | iPSs transplanted into ipsilateral striatum and cortex | No behavioral improvement; tumor formation | 25 |
| | Human early neural progenitors; iPSCs from dermal fibroblasts | Wistar rats; stroke model | Intracerebral transplantation | Engraftment, cell differentiation; no functional improvement | 26 |
| | Human neuroepithelial progenitor cells; iPSCs from dermal fibroblasts | C57BL/6 mice and Nude rat models of stroke | Intracerebral transplantation | Significant behavioral recovery in engrafted mice; grafts survived in rats up to 4 months | 27 |
| | Human neural progenitor cells; iPSCs from dermal fibroblasts | C57BL/6 mouse stroke model | Transplantation into striatum | Engraftment; improved neurobehavioral recovery; no tumor formation | 28 |
tion in immunodeficient \textit{Alb-uPA}^{+/-};\textit{Rag2}^{-/-};\textit{Il2rg}^{-/-} mice (19).

**Neurological Diseases**—Experiments in several neurodegenerative disease models have been reported using neural cells derived from iPSCs. Emborg \textit{et al.} (20) recently reported the application of neural progenitor cells derived from iPSCs in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson disease in rhesus monkeys. Progenitor cells differentiated into neurons, astrocytes, and oligodendrocytes after transplantation and persisted for at least 6 months. These autologous cells induced a minimal inflammatory response, but no functional improvement was reported due to the small size of the graft (20). Rhee \textit{et al.} (21) reported significant motor improvement using reprogrammed and differentiated human iPSCs delivered to rats with striatal lesions. Human oligodendrocyte progenitors generated from iPSCs mitigated symptoms in a rat model of lyssolecithin-induced demyelinated optic chiasm (22). Neural progenitor cells derived from murine or human iPSCs promoted functional and electrophysiological recovery after grafting into the injured spinal cord of rodents and common marmosets, respectively (23, 24). Mixed results have been obtained when either rodent or human iPSC-derived progenitor cells have been transplanted into stroke-damaged mouse or rat brains. Results ranged from tumor development and the absence of any effects on behavior to significant recovery of function, controllable cell proliferation, and formation of electrophysiolgically active synaptic connections (25–28). Among the reasons for variability are the absence of standard protocols for cell preparation and for modeling stroke and testing treatment outcomes. Additional causes of inconsistency include poor cell survival, statistically underpowered animal groups, biological variation, and measurement errors.

**Degenerative Diseases of the Eye**—iPSCs show promise for treating diseases caused by functional defects of the retinal pigment epithelium (RPE), such as age-related macular degeneration, gyrate atrophy, and certain forms of retinitis pigmentosa. Among the advantages for the use of stem cell therapy for these conditions are the immune-privileged character of the target tissue; requirements for limited numbers of cells; and the convenience of monitoring cell injection, potential therapeutic effects, and complications. Protocols have been developed for differentiation of human iPSCs into multipotent retinal progenitor cells and RPE. Retinal function was restored in immunocompromised rhodopsin knock-out (Rho^{-/-}) mice by injection of cells differentiated from mouse iPSCs (29). Swine photoreceptor cells differentiated from iPSCs integrated into the damaged neural retinas of pigs, although significant changes in electroretinal function were not observed, probably due to the limited number of transplanted cells (30). Injection of human RPE cells into the subretinal space of Rpe65^{+/-};Rpe65^{rd12} mice restored vision, including over the long term (31). Future studies of eye disease should develop approaches to support proper transplanted cell integration, including the use of natural and synthetic scaffolds.

**Heart Disease**—Development of the technologies to generate iPSCs and differentiate these cells to functional cardiomyocytes, endothelial cells, and smooth muscle cells is an exciting new development for regenerative medicine (32–35). For human cells, the low original efficiency of differentiation was improved substantially by modifications of the original procedures (36, 37). The potential use of heterogeneous cell populations was explored in rodent ischemic models (7, 37). Injection of cardiac progenitor cells derived from iPSCs into the ischemic rodent heart resulted in functional improvement, although the effect for the most part was temporary due to poor engraftment of the cells. Canine and porcine endothelial cells were generated from iPSCs and used to treat immunodeficient murine models of myocardial infarction (7, 38). Both types of cells improved cardiac contractility by releasing paracrine factors. Alternative approaches have been suggested, such as the use of several distinct heart cell types to regenerate individual components of the cardiac tissue and the use of earlier stage progenitor cells (35, 36). The physiological difference between human and mouse hearts and the dramatically different heart rates present additional problems for use of mouse models. Recently, Templin \textit{et al.} (39) reported vascular differentiation and long-term engraftment of human iPSCs in a pig model of myocardial infarction. The use of human iPSC-derived cardiomyocyte sheets on temperature-sensitive polymers has been explored in the porcine ischemic model in an attempt to improve cell survival and engraftment (40). Additional technological improvements are required to obtain long-lasting therapeutic effects.

**Diabetes**—Reprogramming pluripotent cells to pancreatic β-like cells from a variety of animal species and humans is a critical step in creating an alternative source of insulin-producing cells (41–43). Different stepwise protocols that mimic the process of pancreatic development have been used for reprogramming, but the efficiency of the process is still very low, even using pancreatic β-cells as iPSC precursors (44). Among challenges for differentiation of human cells is the polyhormonal state of a majority of differentiated cells. An insufficient understanding of the regulation of pancreatic development is the major reason that reliable protocols have not yet been developed. Alipio \textit{et al.} (41) reported the application of β-like cells derived from mouse iPSCs for correction of hyperglycemic phenotype in mouse models of type 1 and 2 diabetes. In another study, iPSCs were generated from mouse embryonic fibroblasts and pancreas-derived epithelial cells (45). The latter cell type differentiated more readily to insulin-producing cells. Differentiated iPSCs transplanted into streptozotocin-treated NOD/SCID mice were able to engraft and respond to glucose stimulation by the release of insulin, ameliorating hypoglycemia. Pancreatic progenitor cells also were obtained from rhesus monkey iPSCs generated from adult fibroblasts (42). Treatment of these cells with TGF-β inhibitor led to the generation of insulin-producing cells, which rescued hyperglycemia in streptozotocin-treated diabetic mice.

**Challenges to Be Addressed in Preclinical Studies**

There are many challenges that should be addressed during the process of cell generation and characterization in preclinical studies before clinical application of iPSC-based therapy will be possible (Fig. 1). Cellular imaging within living organisms is expected to play a significant role in evaluating the behavior of transplanted cells or their derivatives. Imaging will provide information about the precise site of cell transplantation; will
guide the accuracy of injection; and will help monitor the number of cells surviving various manipulations and long-term engraftment, cell fate, and therapeutic and off-target effects. The development of noninvasive imaging techniques with high resolution and sensitivity, including deep penetration, will allow in vivo real-time monitoring and help guide human clinical trials (46–48).

Among current concerns for the application of iPSCs are low reprogramming efficiency, the use of reprogramming factors associated with cell proliferation and tumorigenesis, their potential leaky expression, and the use of integrated viral vectors for reprogramming. Technology for the generation of iPSCs is becoming more refined in efforts to address these issues (49, 50). The number, level, timing, and relative stoichiometry of reprogramming factors affect the efficiency, quality, and properties of the iPSCs (51). Other cellular factors and specific pathway inhibitors, as well as noncoding RNA (microRNA and large intergenic non-coding RNA), can affect the process significantly and can increase the efficiency of reprogramming (52, 53). To eliminate the risk of the presence of the transgene used for reprogramming, non-integrating vectors (54), Cre/loxP and piggyBack transposon systems, recombinant proteins, and synthetic RNA-based technologies have been used (53, 55). The efficiency and consistency of these approaches must be improved. Comparison of patterns of the gene expression, epigenetic states, and pluripotent potential of iPSCs with “gold standard” embryonic stem cells from the same species showed that despite almost identical profiles and properties, certain classes of genes and epigenetic marks escape reprogramming in iPSCs (56–59). These differences can be affected significantly by the reprogramming method and by the use of chromatin-modifying drugs.

Some studies have demonstrated low survival and engraftment as well as occasional loss of cell phenotype after transplantation (60, 61). Among different reasons for such behavior is the absence of the proper environment and cell/cell and cell/extracellular matrix interactions in vivo. The use of natural or artificial scaffolds and biologically active molecules developed for tissue engineering and organ reconstruction might help to improve cell retention and survival (62–64). Preclinical studies should address critical issues regarding the ability of the transplanted cells not only to be retained in the target but also to become part of a functional tissue. Genomic mutations represent a serious risk for clinical applications. They should be detected in iPSCs, and their byproducts and mutated cells should not be used. However, it will probably not be possible to prevent all mutational changes. The task is to devise strategies to monitor and evaluate tolerable levels of genetic change and to evaluate the consequences. Numerous studies have compared mutation rates in the original somatic cells and derived iPSCs to analyze at which stage reprogramming affects genomic stability the most (65, 66). The major sources of mutations are carryover aberrations from the original cell source, mutations acquired during cell reprogramming, insertional mutagenesis due to the transgenes used for reprogramming, and passage in cell culture (67, 68). There is a certain preference for accumulation of specific chromosomal aberrations in humans and different animal species. Only certain aberrations are common. Detailed analysis of single-nucleotide changes suggested that most mutations in iPSCs occur during reprogramming and selection of rare mutants in the original cell population (69, 70). Mouse iPSCs were shown to have a significantly lower mutation rate compared with human cells (71). Therefore, there is a need for comparative analysis of cells derived from different species to design preclinical studies to predict the outcome of human trials (72–74).

Epigenomic instability of iPSCs was also reported and is another important property of these cells (75, 76). Several reports indicate the existence of residual specific epigenetic marks from the somatic cells of origin (non-complete repro-
There are significant similarities between cancer cells and iPSCs, which include certain molecular properties, the ability to self-renew, rapid unlimited proliferation, high telomerase activity, expression profiles, and epigenetic signatures (78). As one of the criteria for pluripotency, iPSCs are known to form teratomas in immunocompromised recipients after subcutaneous, intratesticular, or intramuscular injection (79,80). The teratoma-forming capability of the differentiated iPSCs derived from different adult tissues varied substantially and correlated with the number of residual pluripotent cells (81). Importantly, allogeneic transplants in the hearts of immunocompetent rats resulted in tumorigenesis as well (82). Mice generated from tetraploid complementation using iPSC lines were prone to tumorigenesis (83). The expression profiles of the human iPSC-derived differentiated cells revealed a significant overlap of these cells with human tumor cell lines regarding expression of several cancer-related genes (84,85). Because iPSCs themselves are not intended to be used for therapy, the major concern relates to the possible contamination of differentiated progenitors with mutated pluripotent cells. Extensive epigenetic modifications occurring during reprogramming and differentiation may make iPSCs more prone to causing cancer following transplantation. Development of highly sensitive methods for detection and efficient separation of undifferentiated cells will be needed (86,87). Among new methods potentially limiting the tumorigenicity of iPSCs are increasing the copy number of tumor suppressors (88) and the use of specific drugs such as metformin (89) and pluripotent cell-specific inhibitors (90).

There is currently limited information regarding the mechanisms of iPSC-mediated tumorigenesis in vivo. The risk of tumorigenesis is difficult to estimate due to the different susceptibility of animals and humans and to the immunosuppressed or deficient character of the current animal models used in conjunction with human cells. Therefore, additional studies using improved animal models and tests are required. Several new severely immunodeficient mouse and rat models have been developed that will be useful for detecting small numbers of tumorigenic cells in iPSC-derived products (91,92). Tumorigenicity tests should determine the limit of detection and sensitivity of the assay and should contain positive and negative controls. Well defined methods should be developed to reduce the tumorigenicity of transplanted cells, including complete terminal differentiation, eliminating undifferentiated cells, and blocking the expression of cancer-related genes in pluripotent cells and their derivatives. Cancer cells must be detected early after transplantation into the host and eliminated. A sensitive and facile method for tumor detection in small animals is the use of the firefly luciferase reporter construct and bioluminescence imaging (92). However, this approach is not suitable for large animal models and clinical applications. Therefore, technological advances using a combination of imaging modalities are required to provide the most accurate information.

It is important to stress that additional genomic abnormalities can occur during the differentiation of pluripotent cells to specific lineages. Even though the potential risk for tumor formation in these cells should be low, genetic changes can affect their performance and functional activities when replacing damaged tissues (76,93). An additional way to safeguard iPSC-generated cells from overproliferation or teratoma formation after transplantation is to insert inducible suicide genes that can be regulated using prodrugs (94–96).

**Overview of Preclinical Testing Requirements for iPSC Products**

Regulatory issues related to the use of human iPSC products are currently being evaluated by the Center for Biologics Evaluation and Research at the United States Food and Drug Administration (97). According to published requirements, evaluation of iPSC-derived products for patient treatments includes preclinical testing to examine safety, feasibility, and efficacy. Preclinical studies should be conducted and compared in healthy animals and in disease models. In accordance with Food and Drug Administration requirements, the same cells that potentially will be used in humans should be tested in animals. However, for a variety of applications, it will be reasonable at certain stages of development to test cells from the same species to provide a more compatible physiological environment. Rodents are used very successfully for studies of the basic biology of iPSCs, but they are relatively non-predictive for clinical efficacy. Larger animal species such as swine and monkeys may be preferable for stem cell-based preclinical studies due to physiological similarities to humans and longer life spans. It is desirable to develop the surgical and visualization techniques necessary for the use of stem cells in large animals. However, the use of large animals has specific issues that should be considered carefully. Relative to rodents, these include higher cost, more complex husbandry, insufficient reagents and tools, less studied disease mechanisms, less genomic information, a limited number of disease models, and less ability to modify the genome for model development.

In distinction to approved drugs, which have a certain half-life in the body, long-term integration is expected for iPSC derivatives. Cells having a different differentiation status, which can change in response to the in vivo environment, will be potentially present as well. Preclinical studies will involve evaluation of long-term safety and analysis of cell biodistribution. Currently, due to very limited data regarding the fate of transplanted cells, the risk of ectopic engraftment to non-intended locations and long-term off-site effects are uncertain. Therefore, biodistribution studies of stem cell-based products are of primary importance. Among long-term safety issues that should be addressed in preclinical experiments are genomic instability, the immune response and cell rejection, the capacity for uncontrolled proliferation and tumorigenicity, and off-target effects. Testing the feasibility and efficiency of a treatment will have, as an objective, evaluation of biological activity and several clinically relevant outcomes. Preclinical animal testing should provide information regarding biological and behavioral effects in relation to the timing of cell transplantation during the course of the particular disease; the routes for cell delivery; and frequencies, concentrations, and doses of administration. Care should be taken to understand the limitations of extrapolating results obtained in animals to clinical studies, particularly if the organ size, disease mechanism, and pathophysiology are...
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different between the animal model and humans. It is conceivable that, for certain conditions, a single satisfactory model does not exist. Therefore, the use of several models will illuminate potential limitations and enhance the ability to find alternative approaches.

The quality of the cell products, including homogeneity of the cell population, will determine in part the risk and efficacy of a given therapy. Other potentially confounding factors include cell line contaminants, risks of transmissible infections, storage capacity, and viability. iPSC products should be produced according to the protocols and procedures equivalent to Current Good Manufacturing Practice guidelines, and the final products must be characterized thoroughly.

Future Developments and Complementary Approaches

Selection of the best cell sources, further development of effective reprogramming and differentiation protocols, and demonstration of the safety and functionality of specialized cells are urgent issues to be addressed in preclinical studies. Experiments using human and animal cells as model systems will provide unique opportunities to examine a wide variety of functional properties and therapeutic effects in vivo. New strategies for the use of small molecules capable of functionally replacing reprogramming factors and generating tissue-specific precursor cells require further development. The sensitive detection and elimination of potentially tumorigenic cells and the development of appropriate immune models for xenotransplantation experiments, particularly in large animals, should permit more effective translation of experimental approaches to human procedures.

In parallel to the use of reprogrammed iPSCs, the new approach of transdifferentiation, based on the premise of converting one type of somatic cell directly into another, is also attracting considerable attention. This method potentially can significantly shorten the time for obtaining specialized cells and contribute to elimination of the risk of tumorigenesis (98, 99). This new approach requires development of protocols for large-scale production of cells. Problems associated with the lack of complete conversion of one cell type to another must also be solved.

Differentiation of patient-specific iPSCs into the cell types responsible for a given disease potentially provides new in vitro models to study disease mechanisms, test screening tools for toxicology testing, and develop therapeutic drugs to reverse disease phenotypes. Important questions that must be answered are whether cell phenotypes can be discerned within iPSC-derived cell cultures that are representative and predictive of the in vivo pathophysiology underlying the disease of interest and whether this phenotype can be altered in vitro such that a potential therapy for patients can emerge.

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