iPS cells: a game changer for future medicine

Haruhisa Inoue1,2, Naoki Nagata1, Hiromi Kurokawa1 & Shinya Yamanaka1,3,*

Abstract

The induced pluripotent stem cell (iPSC) technology is instrumental in advancing the fields of disease modeling and cell transplantation. We herein discuss the various issues regarding disease modeling and cell transplantation presented in previous reports, and also describe new iPSC-based medicine including iPSC clinical trials. In such trials, iPSCs from patients can be used to predict drug responders/non-responders by analyzing the efficacy of the drug on iPSC-derived cells. They could also be used to stratify patients after actual clinical trials, including those with sporadic diseases, based on the drug responsiveness of each patient in the clinical trials. iPSC-derived cells can be used for the identification of response markers, leading to increased success rates in such trials. Since iPSCs can be used in micromedicine for drug discovery, and in macromedicine for actual clinical trials, their use would tightly connect both micro- and macromedicine. The use of iPSCs in disease modeling, cell transplantation, and clinical trials could therefore lead to significant changes in the future of medicine.

Keywords cell transplantation; cohort study; disease modeling; future medicine; iPSC clinical trial; patient stratification

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See the Glossary for abbreviations used in this article.

Introduction

Like any other scientific advance, the iPSC technology (Fig 1) was established on the basis of numerous findings by past and current scientists in related fields (Yamanaka, 2012). Although the detailed mechanisms underlying the reprogramming process during iPSC generation are still being elucidated, the final products, which had previously been inaccessible, show promise for multiple purposes related to understanding disease mechanisms and strengthening the skills critical for patient treatment (Takahashi & Yamanaka, 2013). Although the iPSC technology still requires improvements and refinement, its contributions to disease modeling and cell transplantation studies are already well-recognized. New technologies, including direct cellular reprogramming and gene-editing, are optimizing the application of the iPSC technology for future medicine.

From this time onward, the progress in iPSCs and associated technologies is expected to engender novel criteria for patient stratification and for the regulation of clinical trials based on drug responsiveness, and the iPSC technology will contribute to more precise medicine in the future.

Disease modeling

The study of disease mechanisms and therapies is being enhanced by iPSC technology-based disease modeling. Following the first report of human iPSCs in 2007, the initiation of iPSC disease modeling was started by the generation of iPSCs using somatic cells from aged patients (Dimos et al, 2008) and patients with many types of diseases (Park et al, 2008), and the variety of diseases being modeled continues to grow (Supplementary Table S1). It is known that drugs used in animal models are not always effective for human beings (Inoue & Yamanaka, 2011). For example, a systematic study of inflammation showed that the gene expression changes in mice had little correlation with the changes seen in humans (Seok et al, 2013). Many genetic variants associated with human diseases are located in non-coding regions that show relatively little evolutionary conservation, which means that their introduction in animals is unlikely to result in phenotypes relevant to human diseases (Merkle & Eggan, 2013). Moreover, it may also be difficult to simultaneously recapitulate the gain and loss of function of the disease-causative proteins in human diseases (Winklhofer et al, 2008) by generating simple transgenic or knockout mice. In addition, one of the statin drugs, compactin, barely reached a human clinical trial level, since it was not effective for rats, in spite of being properly validated in humans (Tobert, 2003). Such discrepancies highlight the significance of using human cells for drug evaluation.

Of prime importance is the establishment of a de facto standard of disease modeling, including the quality control of iPSCs, as shown by previous reports summarized in Supplementary Table S1. However, iPSC disease modeling is faced with several obstacles. It has been revealed that heterogeneous cell populations exist after differentiation from iPSCs, and cells are not able to synchronize the developmental stages of cell populations (Kitaoka et al, 2011). These disparities in the differentiation efficiency and maturation
among clones are considered to originate from incomplete reprogramming, genetic background variability (Soldner & Jaenisch, 2012), epigenetic memory (Kim et al., 2010) or erosion of X-chromosome inactivation (Mekhoubad et al., 2012). There are several points that need to be addressed to overcome these obstacles facing iPSC disease modeling (Table 1), as follows:

Robust differentiation or purification/enrichment of target cells
Using a cell-specific promoter or cell-surface antigen, it is possible to isolate and obtain target cells with the same degree of maturation (Kitaoka et al., 2011; Egawa et al., 2012; Sandoe & Eggan, 2013; Yu et al., 2013), even though perfect purity is not yet possible.

One of the robust differentiation methods is to induce transcription factors for direct differentiation, i.e. direct reprogramming, which can be used to induce specific types of cells, including neurons (Vierbuchen, et al., 2010; Son et al., 2011; Qiang et al., 2013), cardiomyocytes (Ieda et al., 2010), blood cell progenitors (Szabo et al., 2010), hepatocyte-like cells (Huang et al., 2011; Sekiya & Suzuki, 2011) and cartilaginous tissue (Hiramatsu et al., 2011), as well as to determine the germ cell fate (Nakaki et al., 2013). Using this approach, disease modeling is possible (Qiang et al., 2011; Son et al., 2011; Rhinn et al., 2013). The major advantage of the direct cellular reprogramming/induced cell technology is that it works well in large cohorts of samples. On the other hand, there is a limit in the number of original somatic cells used as a resource, meaning that, while the induced cells are suitable for a large cohort analysis, they are not indicated for use in a large-scale analysis using a single line.

Figure 1. iPSC technology contributes to ‘Disease modeling’, ‘Cell transplantation’, and ‘Clinical trial’.
(A) Disease modeling using patient iPSCs for ‘Multi-omics’ and ‘Drug discovery’. (B) Instead of ‘Cell transplantation’, ‘Onsite reprogramming’ may be applied. (C) iPSCs from a large cohort of patients can be applied to ‘Clinical trial’ and ‘Patient stratification’.

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<th>IPSC TECHNOLOGY</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>Disease modeling</td>
<td>Cell transplantation</td>
<td>Clinical trial</td>
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<tr>
<td>Multi-omics</td>
<td>Array analysis of pathways and interactomes</td>
<td>Cellular circuitries</td>
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<td>Genome-wide expression analysis</td>
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<td>Drug discovery</td>
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Glossary
- ALS: amyotrophic lateral sclerosis
- Aβ: amyloid β
- BIP: immunoglobulin heavy-chain binding protein
- ESC: embryonic stem cell
- iPSC: induced pluripotent stem cell
- GSK-3β: glycogen synthase kinase-3β
- HLA: human leukocyte antigen
- p-tau: phospho-tau (Thr231)
- PRDX4: peroxiredoxin-4
- t-tau: total tau
The direct cellular reprogramming/induced cell technology also has advantages in terms of the multi-sample analysis, cost and time, and cellular maturation; iPSCs are preferable in terms of gene-editing, the fact that they are an unlimited resource, and because they can differentiate into a great variety of cells. Although direct cellular programming was revealed to have the disadvantage of not being able to generate a renewable source of programmed cells, several labs have recently shown that programming can be achieved for a proliferating population of neural precursor cells that can then be propagated and subsequently differentiated into mature neurons and glia (Marchetto & Gage, 2012). In addition, the fusion of the direct cellular reprogramming technology with iPSCs would produce a hybrid technology that promotes the merits of both technologies (Imamura & Inoue, 2012), and this has already been reported for neurons (Hester et al., 2011; Zhang et al., 2013), hepatoblasts (Inamura et al., 2011) and myocytes (Tanaka et al., 2013). This hybrid technology will be even more useful after iPSCs can be generated more rapidly, easily and inexpensively.

Mimicking of disease niches by additional conditions

Genetic factors may not manifest functional defects in iPSC models under basal culture conditions, and might require the use of stressors to challenge the cell cultures (Kim et al., 2013). In addition, many neurodegenerative diseases are late-onset diseases, and their key phenotypes may not manifest themselves easily within a short period of time in culture. To mimic the aging process, cellular stress can be imposed, or trophic factors can be depleted.

Selective susceptibility of neuronal cell types in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), can be induced by pathological changes in the neurons, as well as in their interacting partners (Xu & Zhong, 2013).

A highly sensitive detection system

The challenge of elucidating subtle but significant phenotypes in long-term cultures requires the application of multiple complementary readouts. A real-time single-cell longitudinal survival analysis using fluorescent reporter genes has enabled the determination of differences in cellular survival (Bilican et al., 2012). Single-cell expression profiling should clarify the levels of population heterogeneity within in vitro cultures, and advances in media culture platforms and automated cell processing should provide the accuracy and consistency that will be required (Citri et al., 2012; Yu et al., 2013). In addition, specific antibodies against intracellular pathogens have been tested (Kondo et al., 2013), and will continue to be developed.

Optimal control settings

Recent genome-wide association studies have demonstrated that every person possesses disease-relevant single-nucleotide polymorphisms, and it is therefore impossible to categorically define iPSCs that represent perfect non-disease control. Nonetheless, we think that deductive and inductive control (Inoue & Yamanaka, 2011) are valid for deriving iPSC-positive (disease) and negative (non-disease) controls. Deductive controls would include non-disease iPSC/embryonic stem cell (ESC) lines, together with gene-edited, isogenic iPSC lines (see Supplementary Table S1). Many methods have been used in the past for gene targeting in pluripotent stem cell lines (San-doe & Eggan, 2013). Using the isogenic control, a labor reduction and noise cancellation from clonal variations would be possible. On the other hand, multiple clones must be analyzed so as to avoid off-target effects even in the isogenic control lines. If isogenic cell lines are compared, there is no clear answer at present regarding how many isogenic pairs should be analyzed (Merkle & Eggan, 2013).

In addition, when deductive clones are generated by introducing mutations into control human iPSC/ESC lines, protective alleles may intercept the expression of disease-phenotypes.

Validation with human samples and/or other disease models

Although iPSC technology provides novel resources, there is still room for improvement. It is still necessary to better validate the phenotypes with other systems, and to confirm that the phenotypes do not stem from the fragility of the technology by using human samples and other models. In this regard, there are some experimental conditions that only the iPSC technology can provide, such as the co-culture of disease cells and healthy control cells.

Cell transplantation

The iPSC technology is contributing to the study of cell transplantation. The advantages of iPSCs are as follows: Autologous cells, which suppress the risks of rejection and infection, could be used; diseases caused by single gene defects could be addressed by made-to-order gene replacement in cells and allogenic cells from healthy people could be used.

A report of a mouse model of sickle-cell anemia, a genetic blood disorder caused by a defect in the β-globin gene, provided a proof-of-concept illustration of the therapeutic use of iPSCs (Hanna et al., 2007). In that study, a mutant iPSC line with gene correction by homologous recombination was used for transplantation into mutant mice to cure the disease. This exemplified the potential of regenerative medicine using iPSCs (Takahashi & Yamanaka, 2013). It was shown using a non-human primate PD model that autografts caused only a minimal immune response in the primate brain, and autografts have an advantage over allografts even at immunologically privileged sites (Morizane et al., 2013).

In contrast, the use of autologous iPSCs from every individual would necessarily result in high medical costs. Since it takes more than three months to generate iPSCs using the current methods, such a time line is hardly optimal for the effective treatment of certain disorders, such as spinal cord injury (Nakamura & Okano, 2013; Takahashi & Yamanaka, 2013). Furthermore, autografts from sporadic disease cases might harbor disease phenotypes. For these reasons, the importance of considering the use of allogeneic iPSC lines for transplantation therapy must be emphasized. Multiple iPSC clones could easily be generated from the diversity of donor candidates with validated health conditions and the types of human leukocyte antigen (HLA) needed for generating clinical-grade iPSCs.

### Table 1. Points in disease modeling

| 1. Robust differentiation or purification/enrichment of target cells |
| 2. Mimicking of disease niche by additional conditions |
| 3. A highly sensitive detection system |
| 4. Optimal control setting |
| 5. Validation with human sample and/or other disease models |

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clones (Takahashi & Yamanaka, 2013). Matching the three major types of HLA loci between the recipient and donor is expected to result in less immune rejection after transplantation following bone marrow transplantation. One of the most feasible methods for iPSC therapy, therefore, will be based on the collection of iPSC stocks derived from various HLA-homozygous donors under Good Manufacturing Practice (GMP) compliance (Nakajima et al., 2007; Okita et al., 2011; Takahashi & Yamanaka, 2013).

Some new technologies related to cell transplantation have been emerging. Dynamic patterning and structural self-formation of complex organ buds in 3D stem cell culture, including the generation of various neuroectodermal and endodermal tissues, have been discovered (Sasai, 2013). Another example of tissue generation was illustrated by the injection of wild-type rat pluripotent stem cells into the blastocysts of Pdx1-deficient mice, which are unable to grow a pancreas, and this resulted in the generation of normally functioning rat pancreatic tissue (Kobayashi et al., 2010). The self-organization of tissue development, a major advantage of pluripotency-mediated strategies, would be valuable not only for the next generation of organ transplantation, but also for disease modeling (Takahashi & Yamanaka, 2013). Furthermore, on-site reprogramming technology (Yu et al., 2013) has already been applied to the production of β-cells (Zhou et al., 2008) and cardiomyocytes (Qian et al., 2012; Song et al., 2012; Inagawa et al., 2012), and it will progress following the development of improved delivery methods. Immunological cells, including T cells (Nishimura et al., 2013; Vizzard et al., 2013; Wakao et al., 2013), are also expected to be used for cell therapy.

**New iPSC-based medicine**

The iPSC technology has opened new possibilities for generating continuous supplies of progenitor cells for toxicity screening. A toxicity assay using iPSCs would be the first step in clinical trials (iPSC clinical trials). Proof-of-concept toxicity studies performed with human iPSC-derived differentiated cell types (Guo et al., 2011; Medine et al., 2013) support the concept of large-scale human cell-based toxicity screens. Drug-induced side effects in the liver, heart and brain have been thoroughly studied. It is both feasible and effective to use iPSC-derived cells between the drug discovery phase and development phase as clinical trial ‘Phase 0.5’. However, there are several limitations to the sourcing of these cells, such as the achievement of fully mature phenotypes.

While stem cell-based hepatocyte toxicity assays are still at an early stage of development, proof-of-concept studies of known toxicants have been performed (Scott et al., 2013). It was also demonstrated that iPSC-derived cardiomyocytes could be treated using a subset of known arrhythmogenic drugs (Guo et al., 2011; Lahti et al., 2012). Applying electrophysiology methods to study the response of iPSC-derived cardiomyocytes to drug treatment provided prospective results, but such results are limited to the different experimental setups and the number of drugs evaluated in each study has been small (Deshmukh et al., 2012).

The results of these preliminary studies indicate that the toxic compounds that are already well known and have known mechanisms of action should be tested first with iPSC-derived cells, and the requirements, properties and differentiation protocols for the cells derived from standard iPSCs should be decided based on these findings.

In contrast to the drug-induced hepatotoxicity and cardiotoxicity, the mechanisms of which are relatively easy to discern, the reverse-translation of neuronal side effects into discrete cellular mechanisms and toxicity pathways for *in vitro* screening remains a challenge. However, proof-of-concept studies using the high-content analysis of different cell types are expected to be conducted by analyzing the features of neurodevelopment, including neurite outgrowth and synaptogenesis (Scott et al., 2013).

In an aging society, one of the unmet medical needs is that of drug development for Alzheimer’s disease (AD). We previously analyzed the neural cells from AD patient iPSCs, and found that there are subgroups among AD cells. This indicates that clinical AD may need to be reclassified into different sub-types, and that the prediction of the drug responsiveness may be possible based on the different sub-types (Kondo et al., 2013). If we scale up the study, such as by performing an iPSC clinical trial of Phase 1.5, and generate AD and control iPSCs from a larger cohort of patients, it may become possible to select responders and non-responders to specific drugs, leading to a Phase II clinical trial only for responders. Or we could identify a responder marker, after actual clinical trials of a drug, using the iPSCs from responders and non-responders in the trials. This identified marker could then be used to enrich the responders in the next step, leading to higher success rates. Another report also showed that the neurons generated from iPSCs derived from four AD patients showed significantly higher levels of Aβ40 in the culture medium of the neurons generated from three of the four patients, supporting the concept of the heterogeneity of AD (Israel et al., 2012).

There have also been other reports showing patient stratification with the differential drug responsiveness (Table 2). For example, several clinical trials for spinal muscular atrophy (SMA) have been conducted. The completed clinical trials demonstrated that valproic acid (VPA) is only beneficial to a restricted subset of SMA patients, and that there are responders and non-responders (Garbes et al.,

**Table 2. Responder selection in a disease with differential drug responsiveness**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Drug</th>
<th>Marker</th>
<th>Total (n) control/disease/responder</th>
</tr>
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<tbody>
<tr>
<td>Retinitis pigmentosa</td>
<td>α-Tocopherol</td>
<td>RP9 mutation</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/5/2</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>β-secretase inhibitor</td>
<td>Aβ(1-40), GSK-3β, p-tau/t-tau</td>
<td>6</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>VPA</td>
<td>CD36</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/2/1</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>DHA</td>
<td>Aβ oligomer, BiP, PRDX4</td>
<td>7</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>3/4/2</td>
</tr>
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The drug responsiveness of neuronal cells derived from responder iPSCs and non-responder iPSCs to VPA was compatible with the results of the clinical trials (Garbes et al., 2013). Although large clinical trials have been conducted with α-tocopherol (vitamin E), no statistically significant change in visual function of retinitis pigmentosa (RP) patients was found (Jin et al., 2011). The underlying mutations causing the disease in the patients tested in the above clinical trials were not revealed, and the variability of individual responses to these drugs is unknown. However, a recent study showed that the rod cells derived from iPSCs of RP patients showed differential responsiveness to vitamin E, suggesting that RP may be divided into subgroups by the drug responsiveness (Jin et al., 2011). Therefore, the iPSC technology can contribute to micromedicine, including drug discovery based on cellular and molecular analyses, as well as to macromedicine, including patient stratification based on cellular and molecular analyses of participants in clinical trials or cohort studies (Fig 2).

iPSC clinical trials may make it possible to identify a drug-responsive subgroup of patients with a specific disease, and a more precise Phase II clinical trial could thus be performed (Fig 3). The iPSC clinical trial approach could be applied to a large cohort analysis with medical records and genome information. A genome analysis provides ample information, but it is hard to establish sporadic disease models on the basis of such findings. We found that the Aβ metabolisms differed according to the respective APP mutations (Kondo et al., 2013), and an APP mutation that protects against Alzheimer’s disease was recently reported (Jonsson et al., 2012). These findings suggest that, besides the genomic analyses, iPSC-derived cells would be useful for precise analysis of the individual genes and proteins. In addition when a new mutation is found, an analysis of target cells derived from iPSCs would provide an answer to the question of whether the mutation is pathogenic or not (Egashira et al., 2013).

We believe that iPSCs can be game changer that will help to avoid the possibility that a candidate drug tested in a clinical trial might be irrationally dropped based on the old rules. The previous clinical diagnoses are now changing based on the results of the genome analysis and multi-omics analysis of patient samples, including iPSCs. In addition, patients can be stratified based on the drug responsiveness of their iPSC-derived cells, which, as a consequence, could lead to a new type of diagnosis and stratification. A genetic diagnosis of sporadic diseases is difficult, but a drug response-based diagnosis might be possible based on the effectiveness of drugs in clinical trials (Fig 4). The required conditions for iPSCs used in vitro are different from those used for cell transplantation. The development of technologies for generating budget-conscious personalized iPSCs rapidly, homogenously and easily will be required for such iPSC-based clinical trials. To make iPSC clinical trials a reality, the regulatory system would need to be changed.

**Figure 2.** iPSC medicine in drug development ranges from ‘Micromedicine’ to ‘Macromedicine’.
**Figure 3.** iPSC clinical trial for phase 0.5, 1.5, and 2.

**Figure 4.** Patient diagnoses in the past, present, and future.
Conclusions

According to the current medical technologies, after the onset of a disease, patients are diagnosed and treated. Although the significance of the prevention of chronic diseases is well recognized, preventive medicine, which has been developed based on epidemiological studies and statistics, cannot be applied to individuals, and cannot provide a precise diagnosis or individualized therapeutics. Theoretically, everybody has disease-relevant SNPs, and every person has an increased change of becoming a patient during his/her lifetime. The iPSC technology will contribute to personalized, predictive, preemptive (Zerhouni, 2005; Auffray et al., 2009) and precision medicine (Mirmezami et al., 2012).

Supplementary information for this article is available online: http://emboj.embopress.org

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Author contributions

SY: conceived this project. HI: designed the figures and the concepts. SY and HI: wrote the paper. NN and HK: collected and analyzed the data of Supplementary Table S1.

Conflict of interest

S.Y. is a member without salary of the scientific advisory boards of iPienian, iPSC Academy Japan, Megakaryon Corporation and HEALIOS K. K. Japan.

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