Novel Insights into Disease Modeling Using Induced Pluripotent Stem Cells

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Received October 31, 2012

Induced pluripotent stem cell (iPSC) technology has great potential to establish novel therapeutic approaches in regenerative medicine and disease analysis. Although cell therapy using iPSC-derived cells still has many hurdles to overcome before clinical applications, disease analysis using patient-specific iPSCs may be of practical use in the near future. There are several reports that patient-specific iPSC-derived cells have recapitulated the apparent cellular phenotypes of a wide variety of diseases. Moreover, some studies revealed that it could be possible to discover effective new drugs and to clarify disease pathogenesis by examination of patient-specific iPSC-derived cells in vitro. We have recently reported that iPSCs can be a diagnostic tool in a patient with a novel mutation. For definitive diagnosis in a patient with long QT syndrome who had an uncharacterized genetic mutation, we succeeded in clarifying the patient's cellular electrophysiologic characteristics and the molecular mechanism underlying the disease phenotype through the multifaceted analyses of patient-specific iPSC-derived cardiomyocytes. In this review, we focus on the conceptual and practical issues in disease modeling using patient-specific iPSCs and discuss future directions in this research field.

Key words induced pluripotent stem cell; disease modeling; cardiovascular disease; long QT syndrome

1. INTRODUCTION

Induced pluripotent stem cells (iPSCs) are defined as artificial pluripotent stem cells that can be generated from somatic cells by introducing reprogramming factors (e.g., OCT3/4, SOX2, KLF4, c-MYC, NANOG, and LIN28).1,2) The methodology for generating iPSCs has markedly improved and now integration-free iPSCs, without transgene insertion in the host genome, can be obtained using several procedures.3–7) iPSCs maintain the two essential stem cell characteristics of infinite self-renewal capability and pluripotency, meaning that they can give rise to all cell types of the three germ layers and differentiate in a fashion similar to normal embryogenesis.8,9)

One of the expectations of iPSCs is the generation of human disease-specific pluripotent stem cells from patients. Such iPSCs, referred to as patient-specific iPSCs, can differentiate into any type of cell including a patient's diseased organ tissue, and the genetic information of patient-specific iPSCs is identical to that of the patient.10) Therefore we can directly and repetitively analyze diseased cells using patient-specific iPSC-derived cells. Figure 1 shows the conceptual scheme for the utilization of patient-specific iPSCs in clinical practice. To date, many groups have reported that the apparent cellular phenotypes of genetic disorders can be recapitulated in patient-specific iPSC-derived cells in vitro. One of the reports also involved drug screening using iPSCs, resulting in the proposal of novel drug candidates.11,22) We have recently reported that functional analyses of patient-specific iPSC-derived cardiomyocytes elucidated the molecular mechanism of the disease phenotype in a patient with undiagnosed sporadic long QT syndrome (LQTS).13) This paper reviews current topics in disease modeling using patient-specific iPSCs and introduces our study as an actual example in this research field.

2. GENERATION OF PATIENT-SPECIFIC iPSCs

Disease Selection Although any type of disease can theoretically be reproduced by patient-specific iPSC-derived cells, in many diseases it appears difficult to recapitulate the phenotype using this technique because of problems related to both the properties of iPSCs and the disease causality. First, the differentiation efficiency of iPSCs into specific cells restricts the category of disease.4,14) In terms of the maturity of iPSC-derived cells, it may be easier to reproduce the phenotype of disease occurring in younger individuals because of the immaturity of iPSC-derived cells.15) Disease mainly caused by the alteration of epigenetic status due to environmental parameters is not suitable for modeling using iPSCs because the cellular epigenetic information can be partly renewed during the process of reprogramming.16,17) On the other hand, in disease directly caused by a genetic aberration that is clearly preserved in iPSCs, it is feasible to confirm whether patient-specific iPSC-derived cells can reproduce diseased cellular kinetics. In addition, apparent phenotypes can be determined even at the single-cell level because of the difficulty in organ formation from iPSCs.18)

Considering those issues comprehensively, the first disease to be analyzed using patient-specific iPSCs should be a mono-genetic disorder with severe phenotypes diagnosed in infancy and easily examined with simple methods at the single-cell level. Most studies using patient-specific iPSCs focus on diseases that satisfy such requirements. LQTS was selected by
our and other groups as a disease model using patient-specific iPSCs. LQTS is an inherited life-threatening disease caused by functional impairment of the cardiac ion channel with a monogenetic aberration and often causes sudden cardiac death due to ventricular tachyarrhythmia even in infancy.19,20)

Derivation and Characterization of Patient-Specific iPSCs Originally, iPSCs were generated from dermal fibroblasts in a retroviral transduction system.1,21) Subsequently, the methodology for generating iPSCs rapidly improved and became simpler and more efficient, enabling the generation of iPSCs using less patient-invasive methods. Moreover, using plasmid vectors, RNA viruses, and other methods, good-quality iPSCs can be obtained without the need for integrating reprogramming factors.3–7) Integration-free iPSCs appear ideal because exogenous genes integrated in the host genome may affect the genetic properties of the iPSCs generated and modify the cellular phenotypes of patient-specific iPSC-derived cells.22)

We previously reported that integration-free iPSCs can be efficiently, easily, and rapidly generated from terminally differentiated circulating T lymphocytes in peripheral blood using Sendai virus (RNA virus).23) Our method makes it possible to generate iPSCs from any patient including infants, girls, and the very elderly via simple blood sampling, which is one of the least-invasive common clinical procedures. Such cumulative progress in generating iPSCs can accelerate the widespread application of patient-specific iPSC technology.

Before the utilization of generated iPSCs in disease modeling, their characteristics must be evaluated.24) It should be determined whether problems occurred during iPSC reprogramming and maintenance, such as the occurrence of somatic coding mutations,25) dynamic changes in the allelic copy number variation,26) abnormality of X chromosome inactivation,27) incomplete demethylation,28) etc. These elements may affect the phenotype of iPSC-derived cells and skew the interpretation of the results of their assay. In addition, the most appropriate control group remains controversial. In most previous studies, the control groups comprised healthy volunteers without genetic mutations who were unrelated to or relatives of the patients involved. It remains unclear which controls are optimal in disease modeling using patient-specific iPSCs. To examine the unadulterated functions of mutated genes, it appears preferable to compare patients with family members who do not carry the mutation, although related...
family members partly share genetic information including single-nucleotide polymorphisms, and this could affect disease phenotype. A recent study has demonstrated that ideal control iPSCs can be obtained by mutated gene correction using a targeting strategy.\textsuperscript{29,30} Even though it cannot be applied in every disease model, further analysis using isogenic-control iPSCs may be the answer to this problem.

**Differentiation into Disease Relevant-Cells** iPSCs can give rise to a wide variety of cell types present in the three germ layers. In most cases, differentiation methods for iPSCs are applied with some modification from the methods established in embryonic stem cells, which are similar to the regulatory mechanisms of normal early development.\textsuperscript{8,9} To establish methods for in vitro differentiation from pluripotent stem cells, various screening methods for essential signaling molecules in normal development have been performed.\textsuperscript{31}

Among several iPSC lines, the variation in differentiation propensity into specific cell types is well known.\textsuperscript{32,33} Therefore the cell type of each iPSC generated should be confirmed before selecting the optimal cell line that can most efficiently differentiate into the cells of interest. A recent study has shown that iPSCs maintain epigenetic memories originally belonging to somatic cells, and this epigenetic status can regulate the characteristics of iPSCs, especially their differentiation propensity.\textsuperscript{34–36} Therefore it is important to confirm which cells are the best source for iPSCs to obtain stable, disease-relevant cells.

To investigate more sophisticated experimental conditions similar to the physiologic environment, further improvements are required. First, it is necessary to establish a procedure to purify the cells from aggregations of iPSC-derived miscellaneous cells.\textsuperscript{37,38} In addition, it would be ideal to be able to differentiate iPSCs into all constitutive cell types of an organ. In other words, to create organs in vitro, not only a single specific cell type but also other cell types such as endothelial cells, fibroblasts, and peripheral neural cells are needed. Furthermore, there are various subpopulations among cardiomyocytes such as atrial-, nodal- and ventricular-type cardiomyocytes, although at present there is no method to obtain each specific cell type.\textsuperscript{39} These are crucial limitations on the reliability of results of the novel iPSC assay. In addition, iPSC-derived cells retain the original fetal-like characteristics, and it remains unclear how these cells can be appropriately matured.\textsuperscript{40} Still another unresolved issue is the best time in the developmental stage of patient-specific iPSC-derived cells to analyze cellular function in terms of disease properties.

A recent advance in reprogramming to change the cellular fate is direct conversion, which allows terminally differentiated cells to be transformed into other functional cells of different lineages without passing through the pluripotent stage.\textsuperscript{41,42} In this method, mature target cells can be obtained within a shorter period, and disease modeling using this direct conversion technique has also been reported.\textsuperscript{43} However, in spite of lower induction efficiency and the lack of a method established for all cell lineages, iPSCs seem to be a suitable cell source for disease modeling. The infinite self-renewability of iPSCs allows repetitive, reproducible analysis of the disease cells of interest.

### Disease Modeling Using Patient-Specific iPSCs

To date, several patient-specific iPSC lines have been generated from patients with a wide variety of mainly monogenic, early-onset diseases such as neurologic disorders,\textsuperscript{44,45} heart disease,\textsuperscript{46,47} metabolic disease,\textsuperscript{48} hematologic disorders,\textsuperscript{49} mitochondrial disease,\textsuperscript{50} chromosomal abnormalities,\textsuperscript{51} telomere disease,\textsuperscript{52,53} sensory organ disorder,\textsuperscript{54} and storage disease.\textsuperscript{55} The current list of studies of disease modeling using patient-specific iPSCs is shown in Table 1. While findings on patient-specific iPSCs have accumulated, analysis becomes more complicated in polygenic, sporadic, late-onset disease.\textsuperscript{12,56,57}

The next steps that will deliver useful clinical information resulting from patient-specific iPSC technology will result from collaborations between academic research groups and

<table>
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<tr>
<th>Disease</th>
<th>Gene mutation</th>
<th>Cell type</th>
<th>Cellular phenotype</th>
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<tr>
<td>AD</td>
<td>PS1 mutations</td>
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<td>Increase in Aβ/secretion and rescued by γ-secretase inhibitors</td>
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<td>PD</td>
<td>LRRK2 mutations</td>
<td>Neurons-dopaminergic</td>
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<td>CPVT</td>
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<td>Abnormal dynamism in Ca handling and treatment with several drugs rescues the phenotype</td>
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<td>BCR-ABL</td>
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<td>Imatinib resistant in iPSCs and immature Hematopoietic cells</td>
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<tr>
<td>MD (+DM)</td>
<td>mtDNA A3243G mutation</td>
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<td>Variety of degree of mutation heteroplasm in each iPSC clones</td>
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<tr>
<td>DS (+AD)</td>
<td>Trisomy 21</td>
<td>Neurons-cortical</td>
<td>Secretion of the pathogenic peptide fragment amyloid-β42</td>
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</tr>
<tr>
<td>DKC</td>
<td>DKC1, TERT, TCAB1 mutation</td>
<td>iPSCs</td>
<td>Progressive telomere shortening and loss of self-renewal of iPSCs</td>
<td>52, 53</td>
</tr>
<tr>
<td>RP</td>
<td>RP1, RP9, PRPH2, RHO mutations</td>
<td>Rod photoreceptor cells</td>
<td>Decreased numbers of differentiated rod cells and expression of cellular stress markers</td>
<td>54</td>
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<tr>
<td>GD</td>
<td>GCase mutations</td>
<td>Neurons-dopaminergic</td>
<td>Lysosomal protein degradation, causes accumulation of α-synuclein</td>
<td>55</td>
</tr>
</tbody>
</table>

pharmaceutical companies, which are expected develop novel therapeutic compounds and clarify possible side effects through advanced high-throughput screening systems using patient-specific iPSC-derived cells.

3. CARDIOVASCULAR DISEASE MODELING USING iPSCs

Functional Characteristics of iPSC-Derived Cardiomyocytes

On the premise that the study of human cardiovascular disease modeling will be initiated using patient-specific iPSCs, it is necessary to confirm that the characteristics of human iPSC-derived cardiomyocytes are physiologically analogous to human cardiomyocytes in vivo. Previous molecular biological and physiologic studies revealed that iPSC-derived cardiomyocytes have normal cardiomyocyte functional properties.69–77 iPSC-derived cardiomyocytes have a striated muscle structure identical to that of normal functional cardiomyocytes and express cardiac-specific proteins, as confirmed in molecular biological assays such as immunocytochemistry and reverse-transcriptase polymerase chain reaction (PCR). Based on the waveform of the action potential, iPSC-derived cardiomyocytes can be divided into three subpopulations: atrial, nodal, and ventricular cells. Moreover, the contraction of iPSC-derived cardiomyocytes is regulated by physiologic intracellular signaling including excitation-contraction coupling.69 and those cardiomyocytes express typical ion channels with the expected functional responses to several ion channel blockers.69 All these findings indicate the validity of studies that will lead to the analysis of cardiovascular disease using patient-specific iPSC-derived cardiomyocytes.

Modeling LQTS Type 1

Some groups thought that LQTS would be a suitable disease for modeling using iPSCs because of the promising reproducibility of disease phenotypes in iPSC-derived cardiomyocytes.69,72 Moretti et al. first showed that patient-specific iPSC-derived cardiomyocytes could recapitulate the disease phenotype in congenital LQTS.69 They generated iPSCs from two patients with LQTS type 1, who had autosomal-dominant inheritance of a G569A missense mutation in the KCNQ1 gene encoding the IKs current which was previously shown to be relevant to LQTS onset by functional analysis of the mutated gene.69

Individual cardiomyocytes derived from LQTS type 1 patient-specific iPSCs (LQTS1-iPSC-CMs) showed prolonged action potentials using whole-cell patch clamping compared with cardiomyocytes from healthy control donors who were unrelated to the patients. Moreover, LQTS1-iPSC-CMs exhibited increased susceptibility to catecholamine-induced tachyarrhythmia, which is one of the most important clinical features of the syndrome.69 Even though that study was recognized as an important work first confirming the great potential of patient-specific iPSCs, we thought that there was room for expansion of the scope. In not only that study but also in other reports of LQTS disease modeling using iPSCs, patients who had mutated channel profiles characterized by conventional experimental methods were selected. In reality, many patients have unknown mutations that give no specific information on their disease phenotype. To address whether iPSC technology could be used to characterize the disease phenotype with a novel mutated gene, we selected LQTS patients with no family history or previous disease characterization.73

We generated iPSCs from a 13-year-old boy who was a sporadic LQTS patient. He had survived cardiopulmonary arrest due to ventricular fibrillation, and his subtype of LQTS could not be diagnosed using standard clinical tests.69,73 Two healthy volunteers served as controls who donated iPSCs that had differentiated into cardiomyocytes. Our patient had a novel heterozygous mutation located in the KCNQ1 gene, 1893delC, identified by genotyping of his blood sample.72 Electrophysiologic function was measured using a multielectrode array system,73 which showed that the duration of the field potential was markedly prolonged in LQTS-iPSC-CMs as compared with cardiomyocytes derived from controls, which suggested that LQTS-iPSC-CMs maintained the patient’s characteristics and could be successfully reproduced in this assay system.

Next we tried to confirm the responsible channel for the disease phenotype by precise evaluation of several drug responses. We clarified that the IKs channel was functionally impaired and that the IKr channel could compensate for this effect in LQTS-iPSC-CMs with the administration of several potassium current blockers. In general, the IKr and IKs channels work in a complementary fashion in the repolarization of cardiomyocytes, which is known as the repolarization reserve,74 and we confirmed that this mechanism regulated the balance of the potassium current in LQTS-iPSC-CMs. Arrhythmogenic events in LQTS-iPSC-CMs caused by adrenergic stimulation also suggested that the patient’s IK channel was significantly attenuated.70,71 These findings strongly suggested that cardiomyocytes in the patient’s IKs channel were functionally impaired and that the precise diagnosis was LQTS type 1.75 To confirm the dominant-negative role of the KCNQ1 1893delC mutation in IKs channel function, we performed electrophysiologic and histochemical analyses in iPSC-derived cardiomyocytes and found that KCNQ1 1893delC has a dominant-negative effect via a trafficking deficiency.

Importantly, our study demonstrated that iPSCs could be useful to characterize the electrophysiologic cellular phenotype of a patient with a novel mutation. We performed functional analysis of the novel mutation using patient-specific iPSCs, which may support the diagnosis of LQTS type 1. Moreover, this system allowed us to perform several drug administration tests on LQTS-iPSC-CMs, which would be extremely risky to such a patient in clinical practice.76 Therefore patient-specific iPSC technology can be used for drug evaluation and monitoring. At the same time, we were able to clarify the underlying molecular mechanism of the disease phenotype using this assay system.

4. CONCLUSION

Although iPSC technology is an attractive tool for analyzing human genetic diseases, it is clear that technological innovation remains necessary for the utilization of iPSCs in routine medical practice. Disease modeling using patient-specific iPSCs is a novel procedure for analyzing disease. It enables a direct, repetitive approach to diseased cells and has great potential to elucidate novel disease pathogenesis and develop new therapeutic compounds. However, in terms of the effort, cost, and time required in current studies using iPSCs, routine clinical usage is not yet feasible.77 In addition, improvement of the quality of iPSCs and iPSC-derived cells is required
to make disease models using iPSCs more faithful. Some problems such as genetic mutations during reprogramming, incomplete epigenetic reprogramming, and undesired gene expression should also be controlled and standardized. More sophisticated differentiation, maturation, and purification protocols will be indispensable to create physiologic cellular conditions that reflect the actual disease phenotype.

In conclusion, steady progress is being made in iPSC technology to overcome the hurdles, and disease modeling using iPSCs appears a likely technique for the future. Recent and future innovations in the technique hold out the promise of patient-derived iPSC technology to achieve personalized medicine in the clinical setting.

REFERENCES


